Volume 3 September 1951 Number 3

PHARMACOLOGICAL REVIEWS

A quarterly publication in pharmacology, experimental therapeutics, and chemotherapy published for the AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC., under the supervision of the Board of Publication Trustees: M. H. Seevers, *Chairman*, McKeen Cattell, L. S. Goodman, H. B. van Dyke and Arnold D. Welch.

The Editorial supervision of Pharmacological Reviews is shared jointly with the BRITISH PHARMACOLOGICAL SOCIETY and the SCANDINAVIAN PHARMACOLOGICAL SOCIETY.

EDITORIAL BOARD

L. S. Goodman—Salt Lake City, *Chairman*

J. H. Burn—Oxford
J. H. Gaddum—Edinburgh
Thomas C. Butler—Chapel Hill
Alfred Gilman—New York, N. Y.
Harry Eagle—Washington, D. C.
Erik Jacobsen—Copenhagen
U. S. v. Euler—Stockholm
Otto Krayer—Boston
Miles Weatherall—London

CONTENTS

The Nature of Adrenergic Nerve Mediators *by* U. S. von Euler 247

The Influence of Certain Drugs on Blood Coagulation and Related Phenomena *by* Walter H. Seegers 278

Published by
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MD., U. S. A.

PHARMACOLOGICAL REVIEWS

Issued four times a year: March, June, September, and December. One volume of approximately 480 pages per year, subscription price is $6.00 per volume. For countries outside the postal union add $.50 per volume. Single copy price $2.00. Correspondence regarding subscriptions should be addressed to the publishers, or their agents.

Claims for copies lost in the mails must be received within 30 days (domestic). Changes of address must be received within two weeks of the date of issue.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received a pro-rata refund will be made to the subscriber.

To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Journal is not responsible for loss of manuscript in the mail. Authors should retain a copy.

THE WILLIAMS & WILKINS COMPANY
Publishers of Scientific Books and Periodicals
BALTIMORE 2, MARYLAND, U.S.A.

Entered as second-class matter at the post office at Baltimore 1, Md.
Copyright 1951 by the Williams and Wilkins Company
Made in the United States of America

PHARMACOLOGICAL REVIEWS

Issued four times a year: March, June, September, and December. One volume of approximately 480 pages per year, subscription price is $6.00 per volume. For countries outside the postal union add $.50 per volume. Single copy price $2.00. Correspondence regarding subscriptions should be addressed to the publishers, or their agents.

Claims for copies lost in the mails must be received within 30 days (domestic). Changes of address must be received within two weeks of the date of issue.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received a pro-rata refund will be made to the subscriber.

To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Journal is not responsible for loss of manuscript in the mail. Authors should retain a copy.

THE WILLIAMS & WILKINS COMPANY
Publishers of Scientific Books and Periodicals
BALTIMORE 2, MARYLAND, U.S.A.

Entered as second-class matter at the post office at Baltimore 1, Md.

Copyright 1951 by the Williams and Wilkins Company
Made in the United States of America

THE NATURE OF ADRENERGIC NERVE MEDIATORS

U. S. von EULER

The Department of Physiology, Faculty of Medicine, Karolinska Institute, Stockholm

page
I. Introduction 247
II. Historical 248
III. Nature of sympathomimetic substances in adrenergic nerves and in organs with adrenergic nerve supply 249
  a) Adrenergic nerves 249
  b) Organs 251
  c) Release of sympathomimetic substances from isolated nerves 254
  d) Tyramine and hydroxytyramine 254
IV. Release of adrenergic nerve mediators *in vivo* 255
  a) Electrical stimulation of nerves 255
  b) Reflex liberation 258
V. Functional significance of noradrenaline and adrenaline as adrenergic nerve mediators 259
VI. Adrenergic nerve mediators in body fluids 262
  a) Aqueous humour 262
  b) Exudates 262
  c) Various secretions 262
  d) Blood 263
  e) Urine 263
VII. Effect of degeneration and regeneration of the adrenergic nerves on the occurrence of the mediators in organs 265
VIII. Formation of adrenergic nerve mediators 265
  a) Chemical 265
  b) Local 266
  c) Storage 267
  d) Estimation of adrenergic nerve mediator production 267
  e) Differential activation of noradrenaline and adrenaline producing fibres 268
  f) Oxidation products of adrenaline in relation to adrenergic nerve mediators 268
IX. Inactivation and excretion 268
X. Adrenergic nerve mediators in metabolic disorders 269
XI. Adrenergic nerve mediators in invertebrates 270
XII. Adrenergic nerve mediator substances in therapeutic use 270
XIII. Summary 270

I. INTRODUCTION

Our knowledge of the nature of adrenergic (77) nerve mediators, sympathins (64), is founded upon the pioneer research of Loewi (177) and Cannon (68) and their associates.

A number of excellent reviews dealing with this subject are available and should be consulted for detailed information (12, 16, 32, 49, 59, 68, 78, 114, 115, 138, 177, 195, 198, 212). By the recent demonstration of noradrenaline as the chief sympathomimetic agent serving to transmit adrenergic impulses during normal conditions, certain new aspects have been added. An account of the history and development of this concept will be found in more recent reviews (95, 232).

THE NATURE OF ADRENERGIC NERVE MEDIATORS

U. S. von EULER

The Department of Physiology, Faculty of Medicine, Karolinska Institute, Stockholm

page
I. Introduction 247
II. Historical 248
III. Nature of sympathomimetic substances in adrenergic nerves and in organs with adrenergic nerve supply 249
  a) Adrenergic nerves 249
  b) Organs 251
  c) Release of sympathomimetic substances from isolated nerves 254
  d) Tyramine and hydroxytyramine 254
IV. Release of adrenergic nerve mediators *in vivo* 255
  a) Electrical stimulation of nerves 255
  b) Reflex liberation 258
V. Functional significance of noradrenaline and adrenaline as adrenergic nerve mediators 259
VI. Adrenergic nerve mediators in body fluids 262
  a) Aqueous humour 262
  b) Exudates 262
  c) Various secretions 262
  d) Blood 263
  e) Urine 263
VII. Effect of degeneration and regeneration of the adrenergic nerves on the occurrence of the mediators in organs 265
VIII. Formation of adrenergic nerve mediators 265
  a) Chemical 265
  b) Local 266
  c) Storage 267
  d) Estimation of adrenergic nerve mediator production 267
  e) Differential activation of noradrenaline and adrenaline producing fibres 268
  f) Oxidation products of adrenaline in relation to adrenergic nerve mediators 268
IX. Inactivation and excretion 268
X. Adrenergic nerve mediators in metabolic disorders 269
XI. Adrenergic nerve mediators in invertebrates 270
XII. Adrenergic nerve mediator substances in therapeutic use 270
XIII. Summary 270

I. INTRODUCTION

Our knowledge of the nature of adrenergic (77) nerve mediators, sympathins (64), is founded upon the pioneer research of Loewi (177) and Cannon (68) and their associates.

A number of excellent reviews dealing with this subject are available and should be consulted for detailed information (12, 16, 32, 49, 59, 68, 78, 114, 115, 138, 177, 195, 198, 212). By the recent demonstration of noradrenaline as the chief sympathomimetic agent serving to transmit adrenergic impulses during normal conditions, certain new aspects have been added. An account of the history and development of this concept will be found in more recent reviews (95, 232).

Methodological details which take these results into account have been published recently in two reviews (96, 116).

The term *adrenaline* has been used in the following as a synonym for *adrenine* or *epinephrine* in agreement with the opinion of Cannon. In a paper with Lissák (65) he wrote: "... Meanwhile, in foreign languages, and commonly in English writing, the term "adrenaline" has become customary. In accordance with this growing practice that word has been adopted here." Adrenaline also goes well with the generally accepted term "adrenergic." The term *noradrenaline* has been used in the present review as synonymous with *norepinephrine* or *arterenol.* (N.O.R. = N ohne Radikal.)

The occurrence in, and liberation of, noradrenaline and adrenaline from the suprarenal medulla and the chromaffine tissue has been reviewed elsewhere (95, 142) and will be referred to only occasionally in the present review.

II. HISTORICAL

After the first successful demonstration of a chemical transmission of sympathetic nerve effects (70, 176), it was generally believed that the active agent was adrenaline. The discrepancies noted between the effects of sympathetic nerve stimulation and the action of adrenaline (67) then led to the theory of the two sympathins, these agents being supposedly formed by the interaction of a mediator (adrenaline) and certain cell constituents (66, 68). However, doubts as to the validity of this theory were expressed by several investigators (11, 135, 136, 193, 227), and the possibility was pointed out that noradrenaline (arterenol) might be identical with sympathin E and adrenaline with sympathin I. This would also be in accord with the older observations of Barger and Dale (26). However, the findings that postganglionic stimulation of the nerves of the rabbit's ear released what appeared to be adrenaline (118, 120) and that certain organs and their adrenergic nerves seemed to contain adrenaline (65, 174) spoke in favour of adrenaline as a mediator, an opinion which was generally held until 1945.

A closer biological analysis of extracts of sympathetic nerves and of organs innervated by such nerves revealed, however, that the sympathomimetic agent present in such extracts had the characteristic properties of noradrenaline and not adrenaline (86–88).

The previous results of Cannon and Rosenblueth and others (9, 10, 135, 136, 193, 227) could easily be brought in harmony with this finding. Carefully analysed, direct experiments by Gaddum and Goodwin (117) gave further strong support to the concept of noradrenaline as the chief adrenergic mediator. As will be seen in the following, although noradrenaline is the dominant adrenergic chemical transmitter substance, it is not the only one and as pointed out by Bacq (15), is supported by adrenaline and possibly also by other catechol amines. The recognition, for example, that the structural requirements for myocardial stimulation differ from those favorable for sympathetic excitatory or inhibitory action (170) is, in a way, suggestive of other active substances in this group, and so is the finding that so-called adrenolytic substances do not annul the tachycardia provoked by sympathetic nerve stimulation of the heart (200).

Methodological details which take these results into account have been published recently in two reviews (96, 116).

The term *adrenaline* has been used in the following as a synonym for *adrenine* or *epinephrine* in agreement with the opinion of Cannon. In a paper with Lissák (65) he wrote: "... Meanwhile, in foreign languages, and commonly in English writing, the term "adrenaline" has become customary. In accordance with this growing practice that word has been adopted here." Adrenaline also goes well with the generally accepted term "adrenergic." The term *noradrenaline* has been used in the present review as synonymous with *norepinephrine* or *arterenol.* (N.O.R. = N ohne Radikal.)

The occurrence in, and liberation of, noradrenaline and adrenaline from the suprarenal medulla and the chromaffine tissue has been reviewed elsewhere (95, 142) and will be referred to only occasionally in the present review.

II. HISTORICAL

After the first successful demonstration of a chemical transmission of sympathetic nerve effects (70, 176), it was generally believed that the active agent was adrenaline. The discrepancies noted between the effects of sympathetic nerve stimulation and the action of adrenaline (67) then led to the theory of the two sympathins, these agents being supposedly formed by the interaction of a mediator (adrenaline) and certain cell constituents (66, 68). However, doubts as to the validity of this theory were expressed by several investigators (11, 135, 136, 193, 227), and the possibility was pointed out that noradrenaline (arterenol) might be identical with sympathin E and adrenaline with sympathin I. This would also be in accord with the older observations of Barger and Dale (26). However, the findings that postganglionic stimulation of the nerves of the rabbit's ear released what appeared to be adrenaline (118, 120) and that certain organs and their adrenergic nerves seemed to contain adrenaline (65, 174) spoke in favour of adrenaline as a mediator, an opinion which was generally held until 1945.

A closer biological analysis of extracts of sympathetic nerves and of organs innervated by such nerves revealed, however, that the sympathomimetic agent present in such extracts had the characteristic properties of noradrenaline and not adrenaline (86–88).

The previous results of Cannon and Rosenblueth and others (9, 10, 135, 136, 193, 227) could easily be brought in harmony with this finding. Carefully analysed, direct experiments by Gaddum and Goodwin (117) gave further strong support to the concept of noradrenaline as the chief adrenergic mediator. As will be seen in the following, although noradrenaline is the dominant adrenergic chemical transmitter substance, it is not the only one and as pointed out by Bacq (15), is supported by adrenaline and possibly also by other catechol amines. The recognition, for example, that the structural requirements for myocardial stimulation differ from those favorable for sympathetic excitatory or inhibitory action (170) is, in a way, suggestive of other active substances in this group, and so is the finding that so-called adrenolytic substances do not annul the tachycardia provoked by sympathetic nerve stimulation of the heart (200).

III. NATURE OF SYMPATHOMIMETIC SUBSTANCES IN ADRENERGIC NERVES AND IN ORGANS WITH ADRENERGIC NERVE SUPPLY

The demonstration of active substances in the blood leaving an organ or in the bathing fluid of an isolated organ during or after nerve stimulation does not prove, strictly speaking, that these substances are derived from the nerves and serve as chemical mediators. The same criticism may be applied to the evidence based on the occurrence of active agents in extracts of organs. The possibility should be borne in mind that the chemical mediator may appear as an inactive precursor.

From this point of view the demonstration of noradrenaline (87, 89) in adrenergic nerves was particularly clarifying since it not only gave a natural explanation of previous experimental findings as to the effect of stimulation of such nerves but also directly indicated that this substacne was actually the chemical mediator. Inferences as to the adrenergic mediators drawn from the relative activity of certain possible candidates for such transmitters may be misleading (2).

Since a mixture of active substances is generally found in extracts of nerves or organs, and is likely to interfere with the demonstration of specific adrenergic mediators, *e.g.*, acetylcholine and particularly histamine (90, 91), the extracts have to be purified by different methods. These methods have consisted in removal of disturbing agents, for example, by adsorption on fuller's earth (87, 107), or in specific adsorption of the active substances with the aid of alumina or aluminium oxide (90, 182, 206, 223). Anticholinic and antihistaminic compounds have been used to exclude the action of disturbing agents (219). Separation of the active catechol amines may be achieved by countercurrent distribution according to Craig (30), by partition chromatography either on filter paper (160, 161) or on columns (31, 140) or by biological methods (96, 116, 121). Colorimetric methods are generally inapplicable (7, 100), with the exception of Shaw's method (223) and the fluorescence method (45, 171, 182) which require only small amounts of material.

a) Adrenergic nerves

The first evidence of sympathomimetic activity associated with the sympathetic nerves themselves was produced in experiments by Gaddum and Khayyal (115). These authors found that stimulation of adrenergic fibres caused the liberation of a substance acting like adrenaline on the frog's heart. Cannon and Lissák and Lissák (65, 174) made extracts of various nerves containing adrenergic neurones and tested them after dialysis. They reached the conclusion that "the peculiar material which is extracted from sympathetic neurones is adrenaline itself." Part of the evidence was based on the reversal of the effect of the extracts on the cat's blood pressure after ergotoxine. This observation was misleading, however, probably owing to the presence of depressor material such as histamine in the nerve extracts (93, 169).

Subsequently it has been shown that the biological effects of extracts of nerves containing adrenergic fibres agree with those of noradrenaline (87, 89). The extracts were made with acid alcohol and purified with fuller's earth, which

III. NATURE OF SYMPATHOMIMETIC SUBSTANCES IN ADRENERGIC NERVES AND IN ORGANS WITH ADRENERGIC NERVE SUPPLY

The demonstration of active substances in the blood leaving an organ or in the bathing fluid of an isolated organ during or after nerve stimulation does not prove, strictly speaking, that these substances are derived from the nerves and serve as chemical mediators. The same criticism may be applied to the evidence based on the occurrence of active agents in extracts of organs. The possibility should be borne in mind that the chemical mediator may appear as an inactive precursor.

From this point of view the demonstration of noradrenaline (87, 89) in adrenergic nerves was particularly clarifying since it not only gave a natural explanation of previous experimental findings as to the effect of stimulation of such nerves but also directly indicated that this substacne was actually the chemical mediator. Inferences as to the adrenergic mediators drawn from the relative activity of certain possible candidates for such transmitters may be misleading (2).

Since a mixture of active substances is generally found in extracts of nerves or organs, and is likely to interfere with the demonstration of specific adrenergic mediators, *e.g.*, acetylcholine and particularly histamine (90, 91), the extracts have to be purified by different methods. These methods have consisted in removal of disturbing agents, for example, by adsorption on fuller's earth (87, 107), or in specific adsorption of the active substances with the aid of alumina or aluminium oxide (90, 182, 206, 223). Anticholinic and antihistaminic compounds have been used to exclude the action of disturbing agents (219). Separation of the active catechol amines may be achieved by countercurrent distribution according to Craig (30), by partition chromatography either on filter paper (160, 161) or on columns (31, 140) or by biological methods (96, 116, 121). Colorimetric methods are generally inapplicable (7, 100), with the exception of Shaw's method (223) and the fluorescence method (45, 171, 182) which require only small amounts of material.

a) Adrenergic nerves

The first evidence of sympathomimetic activity associated with the sympathetic nerves themselves was produced in experiments by Gaddum and Khayyal (115). These authors found that stimulation of adrenergic fibres caused the liberation of a substance acting like adrenaline on the frog's heart. Cannon and Lissák and Lissák (65, 174) made extracts of various nerves containing adrenergic neurones and tested them after dialysis. They reached the conclusion that "the peculiar material which is extracted from sympathetic neurones is adrenaline itself." Part of the evidence was based on the reversal of the effect of the extracts on the cat's blood pressure after ergotoxine. This observation was misleading, however, probably owing to the presence of depressor material such as histamine in the nerve extracts (93, 169).

Subsequently it has been shown that the biological effects of extracts of nerves containing adrenergic fibres agree with those of noradrenaline (87, 89). The extracts were made with acid alcohol and purified with fuller's earth, which removed most of the depressor material. The conclusion that the chief active agent was noradrenaline was based on the following evidence: a) the pressor effect was diminished or even abolished by sympatholytic substances but not reversed as is that of adrenaline; b) the weak relaxing effect on the non-pregnant cat's uterus; c) the weak pupil dilating action; d) the weak fluorescence in ultraviolet. In all these respects there was good agreement between the action of the nerve extracts and those of noradrenaline, when equi-pressor amounts on the cat's blood pressure were compared, but marked differences from those of adrenaline.

The naturally occurring noradrenaline was identified as the laevo-isomer by comparing the biological activity and the strength of the colorimetric iodine reaction of nerve extracts and *dl*-, and *l*-noradrenaline (89). By such tests good agreement was found only with the *l*-noradrenaline, available just at that time (233, 235).

Although chemical evidence that the predominant adrenergic substance is *l*-noradrenaline is still lacking there is little doubt of their identity on the evidence given above. Further support accrues from the fact that *l*-noradrenaline has been isolated from suprarenal extracts (30) and from commercial non-synthetic "adrenaline" (236), indicating that it does occur biologically.

Noradrenaline has been demonstrated in sympathetic chain and ganglia, postganglionic fibres to various organs, including grey rami, splanchnic nerves and sensory nerves to the skin. It has been found as the predominant adrenergic nerve substance in such nerves from all vertebrate animals examined. Particularly high amounts have been found in the splenic nerves from various animals, which consist largely of unmyelinated postganglionic adrenergic fibres. After removal of the sheath the noradrenaline content of the cow's splenic nerves was found to reach figures as high as 20 µg. per gram (89).

In some cases methods have been used which do not identify the "adrenaline-like" material obtained in extracts of nerves (180, 208).

Table I gives the content of noradrenaline found in various nerves (95). The figures have been compiled from various sources (19, 95, 150, 174).

Information is still lacking as to whether noradrenaline acts as an adrenergic nerve mediator in fish, amphibia and invertebrates.

Closer analysis has revealed that extracts of adrenergic nerves contain, besides noradrenaline, small amounts of adrenaline (94). This evidence is based on the use of methods for differential estimation of noradrenaline and adrenaline (61, 96, 116, 121). For this purpose the action of the extract is tested biologically on two preparations having a sufficiently wide difference in activity ratio between the two catechol amines, such as the blood pressure of the cat and the isolated hen's rectal caecum or the rat's uterus. Other preparations which have been used include the rat's colon, the innervated and denervated cat's spleen, the rabbit's ear (122), the rabbit's or cat's intestine *in situ* (50, 155), and the cat's uterus *in situ* (155). In raising the cat's blood pressure the action of noradrenaline is 1–5 times that of adrenaline, whereas its effect on the rat's uterus may be as little as $\frac{1}{300}$ that of adrenaline. Other test preparations are intermediate. If the activity of the extract on an appropriate pair of preparations is evaluated in terms of noradrenaline and the activity ratio of adrenaline to noradrenaline is estimated on each preparation, the amounts of noradrenaline and adrenaline can be calculated according to a simple formula (56, 94).

TABLE I

Amount of l-Noradrenaline in μg. per Gram of Nerve

NERVE	ANIMAL SPECIES						
	Cattle	Horse	Sheep	Dog	Cat	Rabbit	Man
Brain	0.04–0.2		0.08			0.2	
Spinal cord	0.12						
Vagus	0.1			4	0	0	
Cervical sympathetic	0.6			1	0		
Sympathetic trunk	2.5–4.9	1	3.8	10, 12	12		2–3
Splanchnic	4						
Splenic	8.5–18.5	1.75	8	10–15			
Mesenteric	1.5–3		3.5	20, 18	20	1	
Phrenic	0.15–0.25			0	0	0	
Sciatic			0.14	2.4	4	3.2	
Saphenous	0.2–1						
Superior cervical ganglion	1				16	14	

A = μg noradrenaline equivalents per gram tissue, on hen's rectal caecum.
a = μg noradrenaline equivalents per gram tissue, on cat's blood pressure.
Q = activity ratio adrenaline/noradrenaline, on hen's rectal caecum (>1).
q = activity ratio adrenaline/noradrenaline, on cat's blood pressure (<1).

$$\text{Adrenaline } \mu\text{g./gram} = x = \frac{A-a}{Q-q}$$

$$\text{Noradrenaline } \mu\text{g./gram} = y = A - xQ$$

$$\text{Relative amount of adrenaline} = \frac{x}{x+y} \text{ (degree of methylation)}$$

By use of such biological methods small amounts of adrenaline have been found in nerve extract preparations. Table II gives some examples (98).

Quantitative separation of adrenaline and noradrenaline in nervous tissue extracts by partition chromatography (31, 140) do not seem to have been made. Paper chromatography of splenic nerve extracts have not indicated the presence of catechol amines other than noradrenaline and adrenaline (98).

b) *Organs*

A number of earlier investigations have been concerned with the demonstration in organ extracts of substances having the properties of sympathomimetic amines, biologically (65, 84, 178, 179, 217, 243) or chemically (10, 178, 179, 204–

208, 223). In many of these, evidence for adrenaline-like actions has been brought forth.

By the use of more precise preparative and analytical methods differences between the active organ substances and adrenaline were detected and agreement was found with noradrenaline, as in the case of nerve extracts (86, 88). In confirmation of this, subsequent authors found that the activity in most organ extracts was in all probability chiefly due to noradrenaline (14, 19, 95, 109, 127, 209, 219, 238).

One organ, the placenta, lacks nerves and also sympathomimetic activity (87, 219). Denervation of an organ deprives the extracts of activity (65, 98, 110, 130) and regeneration of degenerated nerves restores it (130). It is therefore likely that the extract activity is due to the nerve mediators present in the nervous tissue. As only adrenergic nerves have been found to contain sympathomimetic substances the available evidence points to the adrenergic nerves as bearers of the corresponding activity of organs.

Estimations of the content of catechol amines in extracts of various organs with the arsenomolybdate colorimetric method of Shaw have indicated that, whereas in some organs practically all of the colour giving material may be adrenaline, other organs contain catechol amines of other kinds (206, 223), judging from the degree of colour increase on adding alkali. A low denominator of the specific ratio (s.d.r.) of colour increase, indicating substances other than adrenaline and possibly identical with noradrenaline, was found with spleen, brain, liver, kidney and heart (rat) (206). In rabbit's heart a higher s.d.r. was found and a still higher one in frog's heart (223). The results obtained with frog's heart are in good accord with the biological estimations (87) and the demonstration of adrenaline by the fluorimetric method (178, 179).

TABLE II

	l-ADRENALINE	*l*-NORADRENALINE	% ADRENALINE
	μg./gram	*μg./gram*	
Splenic nerves, cow	0.09–0.28	7.5–9.4	1.1–3.5
" " sheep	0.1–0.56	5.0–7.7	2–7
Sympathetic chain, man	0.13–0.33	2.4–3.4	5–9
" " sheep	0.06–0.08	1.0–3.8	2–8
Mesenteric nerves, sheep	0.1–0.21	1.2–3.4	6–8

From biological and chemical estimations it appears that the absolute as well as the relative contents of noradrenaline and adrenaline show wide differences in various organs. Differences in the absolute amount of catechol amines may be explained in a natural way, assuming that they are due to variations in the number of adrenergic fibres reaching the organ. Thus the number of such fibres innervating the spleen far exceeds the number in the corresponding nerve supply to the liver, the heart, and the kidney per unit weight of organ. A similar difference holds for the amounts of noradrenaline found in these organs in all animals thus far examined (95). Estimations of the amount of adrenaline as well as noradrenaline in various organs have been made by biological analyses on two test preparations as outlined above, and some of the results are presented in Table III (98, 130).

The predominance of noradrenaline is clearly brought out by the table. The relative adrenaline content is low in spleen and liver and highest in the heart and submaxillary gland. No relation has been found between the total catechol amine content and the adrenaline percentage. The low percentage of adrenaline in the liver is noteworthy in view of the effect of stimulation of hepatic nerves (68, 70, 117, 135), the sympathin of which has been described as "purely excitatory."

No direct evidence is so far available regarding the relative amounts of adrenaline in the nerves innervating striated muscle or in this tissue itself. The larger increase in heat production in the innervated muscle than in the sympathetically denervated muscle after heat puncture (27) would speak in favour of a relatively high percentage of adrenaline, which has a much stronger effect on metabolic processes in general than noradrenaline.

TABLE III

ORGAN (SHEEP)	NORADRENALINE	ADRENALINE	ADRENALINE, %
	μg./gram	*μg./gram*	
Spleen	3.0–3.3	0–0.11	0–3.4
Parotid gland	0.5–2.2	0.03–0.19	5–14
Submaxillary gland	0.4–1.1	0.10–0.21	12–21
Heart	0.6–1.1	0.1–0.2	10–20
Kidney	0.4–0.6	0.05–0.07	11
Liver	0.15–0.20	0.007–0.011	4–7
Lung	0.08–0.1	0.002–0.01	2.5–10

The demonstration and estimation of the typical mediator substances in organ extracts have another interesting consequence; it allows certain conclusions as to the adrenergic nerve supply to an organ. In the brain, for example, the catechol amine content is very low (150) and may only correspond to the vascular supply which includes vasoconstrictor nerves (48).

The significance of the presence of adrenaline in some of these extracts may be discussed from two points of view. Firstly, the adrenaline may be derived partly from chromaffine cells in the organ, and therefore not belong to the adrenergic nerve mediators in the strict sense of the word. Whether such chromaffine cells, like phaeochromocytomas (83, 125, 148), contain mostly noradrenaline or mostly adrenaline is unknown. However, the results obtained with extracts of the prostate gland, which contains chromaffine cell groups, suggest that adrenaline is present in this organ (75, 85, 223). Furthermore, in the superior cervical ganglion the chromaffine cells seem to contain adrenaline (55). Secondly, part of the adrenaline-like activity as determined biologically may be contributed by other catechol amines, such as hydroxytyramine. This catechol derivative which occurs regularly in urine (101, 152) has also been found in extracts of heart (128) and suprarenals (129). Since it may be more active compared with noradrenaline on the isolated hen's rectum than on the blood pressure of the cat, it may simulate adrenaline when extracts are tested on these preparations. On the other hand the amounts of hydroxytyramine found in chromatographic analysis of heart extracts do not account for more than a small fraction of the adrenaline found by the biological method. Other catechol derivatives have so far not been demonstrated. Accordingly, there is good reason to believe that the adrenaline figures represent, at least approximately, the true amounts of adrenaline. Again, the proportion of adrenaline which is due to the presence of chromaffine cells in the organ is harder to evaluate. A contribution to this question has been made by Goodall (130) who found that, in the sheep's heart, the adrenaline content diminished at an even faster rate than that of noradrenaline upon sectioning of the sympathetic nerves. After regeneration the adrenaline content rose again. There is no evidence for a regeneration of preganglionic fibres contacting chromaffine cells left in the organ, so one must assume that the reappearance of catechol amines is due to the ingrowth of postganglionic fibres. It seems reasonably safe, therefore, to assume that the adrenaline found in organ extracts is derived from the adrenergic nerves in those cases where it disappears on denervation.

c) *Release of sympathomimetic substances from isolated nerves*

Several authors have reported that isolated nerves containing adrenergic fibres release adrenaline-like substances on electrical stimulation (8, 63, 99, 173). The significance of such results has been questioned (119) on the ground that the release may be due to damage to the nerve. Although the amounts released are very small and the results rather dependent on the experimental conditions (freshness of nerve, freshly cut surface, etc.), the evidence for such a release seems fairly satisfactory although the physiological significance of the release is doubtful. The results may be of interest for the elucidation of the mechanism of release at the nerve endings, which is not yet understood. Possible mechanisms seem to be increased permeability of the nervous tissue or, rather, a shift in the ion balance causing a release from a complex compound.

d) *Tyramine and hydroxytyramine*

Both tyramine and hydroxytyramine (3,4-dihydroxyphenylethylamine) might come into consideration as supplementary adrenergic nerve mediators, since they exert definite although comparatively weak sympathomimetic actions. A conclusive account of their possible roles in this connection cannot be given at present for lack of experimental evidence. Even if it should be demonstrated that these substances are liberated on adrenergic nerve stimulation, they would probably have a rather limited scope of action.

Whether tyramine occurs as a natural constituent of organs is still a matter of dispute. It is known, however, that it is formed during bacterial decomposition and possibly also in nonbacterial anaerobic autolysis of tissues by decarboxylation of tyrosine (108, 149). In invertebrates tyramine has been found in the salivary secretion of cephalopods (144) and has been considered as a chemical mediator for the cardiomoderator nerves (114). The peculiar observations that low concentrations of adrenaline increase the vascular actions of tyramine and that of nerve stimulation (57, 58, 62, 197) may have physiological importance, and have led to the theory that tyramine acts on the nerve endings and liberates sympathin. Gaddum and Goodwin (117) concluded from their experiments that liver sympathin obtained by hepatic nerve stimulation in the cat might be noradrenaline or perhaps tyramine. Although there is little doubt that noradrenaline is the chief constituent of liver sympathin, an admixture of tyramine cannot be excluded. Further experiments are needed to elucidate this problem, especially since it has been shown that ortho-tyramine and meta-tyramine, which both have effects very similar to those of tyramine, are formed by the action of the enzyme *l*-DOPA-decarboxylase (42) which does not act on tyrosine.

The case for hydroxytyramine as a possible adrenergic mediator is perhaps stronger, since, like noradrenaline and adrenaline, it is present in urine normally and also in organ extracts such as those from the heart and the suprarenals (101, 128, 129, 152). The circulatory effects of hydroxytyramine are weaker than those of adrenaline, the pressor effect in the cat being estimated as $\frac{1}{50}$ to $\frac{1}{100}$ that of *l*-noradrenaline (4, 98).

The formation of hydroxytyramine from DOPA has been demonstrated with renal tissue or suitable extracts (151), and also in the kidney itself under anaerobic conditions (37). Further investigations are needed to ascertain whether any actions of adrenergic nerve stimulation may be explained by the liberation of hydroxytyramine. Its possible role in the formation of noradrenaline and its formation by the decarboxylation of dihydroxyphenylalanine (see p. 266) make a closer analysis of this point desirable.

IV. RELEASE OF ADRENERGIC NERVE MEDIATORS IN VIVO

a) Electrical stimulation of nerves

The critical discussion by Barger and Dale (26) on the effects of adrenaline and of sympathetic stimulation gave the first intimation that the adrenergic nerve mediator differs from adrenaline in its action. The results of Cannon and his coworkers clearly showed that the active agents liberated into the blood stream after electrical stimulation of sympathetic nerves differed, to a greater or lesser extent, from adrenaline. Critical discussions of the theory of Cannon and Rosenblueth concerning the nature of what was believed to be the mediator and the final active products called sympathins I and E will be found elsewhere (11, 95, 117, 135, 136). The bulk of available evidence is in favour of the concept adopted by several investigators (11, 17, 92, 117, 135, 136) that the remote effects observed after stimulation of adrenergic nerves are due to noradrenaline or variable mixtures of noradrenaline and adrenaline. So far no points have been brought forward which are incompatible with such a view.

The evidence for noradrenaline being released by adrenergic nerve stimulation may be summarized as follows:

1. Stimulation of hepatic nerves produces effects on the blood pressure, on the cat's uterus, and on the iris which correspond to those of noradrenaline (117, 135).
2. The rise in blood pressure caused by hepatic nerve stimulation is weakened but not reversed by ergotoxine (66).
3. Adrenergic nerves contain noradrenaline (19, 87, 89).
4. Venous blood from an organ contains noradrenaline after stimulation of its sympathetic nerves (203).

The evidence for adrenaline is of a similar character (66, 94, 189, 203, 241). In some earlier experiments on splenic stimulation, the results would not allow discrimination between adrenaline and noradrenaline (20, 218). The demonstration of noradrenaline and adrenaline in adrenergic nerves seems to make assumptions of a special mediator and secondary active agents superfluous.

If one adopts the term *sympathin* to designate the active group of substances liberated on adrenergic nerve stimulation it becomes advantageous to characterize the various components. An attempt in this direction has been made by the introduction of the terms sympathin N and A for noradrenaline and adrenaline, respectively (92). Any additional component could then be marked by an appropriate suffix. Whether the term sympathin should be used to include the adrenal medullary secretion ("adrenal sympathin" [242]) is highly questionable.

TABLE IV

NERVES	EFFECTS OF STIMULATION		REFERENCES
	Noradrenaline-like	Adrenaline-like	
Hepatic (cat)	+++	–	68, 117, 136, 189
Splanchnic (cat)	+++	+	68
Abdominal sympathetic trunk (cat)	+++	(+)	
Duodenal (cat)	+++	+	
Sympathetic tail nerves (cat)	+++	(+)	
Sciatic	+++	(+)	12, 186, 187
Hypogastric	+++	(+)	
Splenic (cat)	+++	(+)	203, 189
Cardiopulmonary (cat)	+++	(+)	68

Parentheses indicate slight or inconstant action.

In those cases where a differential analysis of the remote effects of sympathetic nerve stimulation has been made possible by observations on more than one organ it has been possible to gain an idea of the nature of the adrenergic mediators in various regions. Table IV is an attempt to correlate the results in terms of noradrenaline and adrenaline.

Especially illuminating were the experiments in which liver sympathin was produced by stimulation of hepatic nerves (117, 135). In these cases a direct comparison of the results of stimulation with those of noradrenaline showed good agreement. Further evidence has been adduced by analysis of hepatic vein blood from cats after stimulation of the hepatic nerves. Noradrenaline was found to be present in the surprisingly high mean concentration of 0.48 μg. per ml. and adrenaline was found only in small amounts (189). Taking this evidence together with the finding of noradrenaline as the dominant sympathomimetic agent in liver extracts (98), there is every reason to believe that this substance actually is responsible for the effects observed and thus serves as the chief adrenergic nerve mediator in the liver.

Since there is not complete similarity between the effect of either adrenaline or noradrenaline and that of stimulating certain adrenergic nerves, the explanation may be advanced that a mixture of the two substances is liberated. As already stated, most other organs, with the exception of the spleen, contain a greater proportion of adrenaline than the liver.

It seems, therefore, that the present evidence is strongly in favour of the assumption that in those organs which contain predominantly noradrenaline this substance also serves as the adrenergic nerve mediator, and that in organs containing a higher proportion of adrenaline, a mixture of noradrenaline and adrenaline is released. A detailed analysis, extended to several organs, concerning the kind of remote effects obtained by sympathetic nerve stimulation compared with the contents of noradrenaline and adrenaline in these nerves and the corresponding organs would, however, be desirable.

It should be possible to draw conclusions as to the nature of the adrenergic transmitter substances of a given organ simply by observing the effect on the organ of stimulation of its nerves, if the organ responds differently in kind to adrenaline and noradrenaline. The following examples of such organs have been reported. The rabbit's uterus in early pregnancy is inhibited by noradrenaline and stimulated by adrenaline (87). After partial postganglionic denervation of the nictitating membrane in the cat adrenaline and nerve stimulation had different effects (165). Adrenaline inhibits the isolated rectal caecum of the hen whereas noradrenaline in the same concentration (10^{-9}) is said to have a motor effect (132). Even in the nonpregnant cat it has been found that occasionally noradrenaline contracts and adrenaline relaxes the uterus freed from its nervous connections. During carotid artery occlusion uterine constriction has been observed, indicating noradrenaline rather than adrenaline as the mediator (133). The response of vessels in skeletal muscle might also be used (3). Nerve stimulation of the rat uterus was found always to simulate the noradrenaline response, whether the uterus was in a pregnant or non-pregnant state (186).

In some cases there is apparently little difference in action between noradrenaline and adrenaline. For example, the demarcation potential of mammalian skeletal muscle is affected similarly by noradrenaline and adrenaline (53), as well as by stimulation of the adrenergic fibres to this muscle, there being an increase up to 10 per cent.

Chronaximetric investigations have indicated certain changes in the content and liberation of nerve mediators from organs but do not seem to permit conclusions as to their nature (72). It is not known whether noradrenaline acts like adrenaline in increasing the salivary flow induced by acetylcholine or chorda stimulation (29).

A problem of particular interest is whether adrenaline is the adrenergic nerve mediator in organs in which this compound is more active than noradrenaline in producing the normal response to nerve stimulation. Such an organ is the intestine, although the difference between the responses to the two agents is not very large. The uterus and the iris show a more marked difference in response to noradrenaline and adrenaline. Adrenaline inhibits the non-pregnant uterus in much smaller doses than noradrenaline. It was therefore of interest that blood from the ovarian vein of a non-pregnant cat contained about 88 per cent of noradrenaline and only 12 per cent of adrenaline on stimulation of the hypogastric nerves (186). Similar results were obtained on the rabbit's uterus (187). On the other hand noradrenaline is a more powerful stimulator of the pregnant uterus than adrenaline (87, 239). One is therefore tempted to believe that the "choice" of the chemical mediator is largely determined by the effect on the organ in its most important functional state. Another question of interest is whether adrenaline serves as the chief adrenergic nerve mediator generally in some species, for example, the rabbit. Attention is called to the fact that the mesenteric nerves of the rabbit contain chiefly noradrenaline (95). The conclusion of West (242) that "in the rabbit adrenaline is more important than noradrenaline in the transmission of autonomic nerve impulses" is not justified and is at variance with the results of the same author (188).

b) *Reflex liberation*

Although it is reasonable to assume that the adrenergic nerve mediators liberated by electrical stimulation would be similar in kind to that produced by natural processes, such as reflex activity, the effects of the latter should be considered separately. A convenient way of producing strong reflex activity in the cardiovascular system is by eliciting the sinus or aortic pressor reflexes by lowering the pressure in the carotid sinus. Reflex activation of the sympathetic system has also been elicited, for instance, by stimulation of the central stump of the cut sciatic (52). When the effect is studied in the whole animal the participation of the adrenals should be taken into consideration. An increased secretion from these glands has been repeatedly shown as a result of carotid clamping, and the relatively high adrenaline content of the medullary secretion of many animals may lead to confusion.

Folkow and Uvnäs (112) produced vasoconstrictor discharge to the hind limbs of a cat having separate circulation in the cranial and caudal parts, by means of carotid occlusion, injection of acetylcholine, asphyxia, CO_2 or stimulation of the central stump of the brachial nerve. Injection of adrenaline or noradrenaline in the lower half of the animal, or stimulation of the sympathetic chain at L5 produced vasoconstriction in the hind limbs. After dibenamine in a dose of 20 mg./kg., adrenaline produced vasodilatation whereas the action of noradrenaline was abolished. On clamping the carotids under these conditions vasoconstriction of the hind limbs no longer occurred, nor did the vasomotor reflex discharge or noradrenaline produce dilatation. The effect of sympathetic stimulation was one of vasodilatation. These results show that the reflex vasoconstrictor action is mediated by a substance differing from adrenaline and behaving like noradrenaline. CO_2 and asphyxia also produced similar effects. The possibility that electrical stimulation of the sympathetic chain produces vasodilatation after dibenamine or ergotamine by releasing adrenaline at the nerve endings seems to be ruled out by the observation that atropine annuls this effect (113). The vasodilatation therefore is interpreted as being due to stimulation of cholinergic vasodilator fibres running in the sympathetic nerves.

The results quoted above seen to show that reflexly elicited vasoconstriction is mediated by noradrenaline and not by adrenaline. However, extracts of hind limb vessels of the cat contain small quantities of adrenaline (98) and it is therefore possible that small amounts of adrenaline are liberated with the noradrenaline, although analysis of the vasomotor action so far has failed to reveal this. Experiments on the perfused leg of the dog (36) suggest a reflex vasodilator action not mediated through cholinergic nerves. (Also see 54, 76, 82, 100, 137, 145, 184, 190, 192, 204, 213, 214, 219, 229–231.)

Differences in action on the renal vessels have been observed after adrenaline and muscular exercise. Since adrenaline appears to constrict the efferent glomerular vessel and exercise causes a constriction of the afferent vessel it is concluded that adrenaline plays no significant role in the renal changes seen in exercise (23). It would obviously be of interest to compare the effects of adrenaline and noradrenaline in this respect.

On the isolated rabbit's ileum stimulation of the mesenteric nerves regularly causes inhibition (6); noradrenaline also causes inhibition (98), but adrenaline often produces inhibition which is followed by stimulation (71).

Here also should be mentioned the release of what has been considered to be adrenaline from the perfused heart by means of acetylcholine, presumably by an action on sympathetic ganglia in the heart (147, 185, 194). The experimental data do not exclude noradrenaline as the chief active substance in the perfusate, although some evidence makes it probable that adrenaline is present in the mixture.

As stated above, the effects of carotid reflexes resemble those of noradrenaline although it should be borne in mind that part of the effect is due to adrenal medullary secretion (50, 131, 155). In such experiments it also has been noted that in the beginning of an experiment noradrenaline predominated but that the proportion of adrenaline gradually increased (51). After asphyxia and hemorrhage the sympathin released from the dog's spleen was found to be increased (225). From the information as to the increased output chiefly of noradrenaline during muscular exercise one can assume that this sympathin originates chiefly from the adrenergic neurones and not from the suprarenals (103). More experiments will be needed to ascertain the kind of adrenergic nerve mediator in various forms of stress (222).

V. FUNCTIONAL SIGNIFICANCE OF NORADRENALINE AND ADRENALINE AS ADRENERGIC NERVE MEDIATORS

If the organism avails itself of two adrenergic nerve mediators or possibly more it would seem likely *a priori* that they differ not only in minor forms of action but in a more fundamental way. Thus the scope of adrenergic activity would be enlarged.

Examples of such differentiation of action of chemically near related compounds are at hand both in the internal secretion of the ovary (oestrone and progesterone) and in the suprarenal cortex, where alterations in the hormone molecule impart striking differences in action. The unitarianism of the "adrenaline era" has given place to a dualism in regard to adrenergic nerve mediators, and it is not improbable that further research will reveal a still more complete situation.

Although it was noted as far back as 1906 (35) and repeatedly confirmed that noradrenaline was considerably less toxic than adrenaline (156, 233), only in the last few years have investigations brought to light a number of important differences in the actions of adrenaline and noradrenaline. It would seem fruitful to try to arrange these differences in a system, although it is certain to suffer from incompleteness at this early date and no doubt will require future modifications.

The rather weaker activity of noradrenaline in comparison with adrenaline on a number of smooth muscle organs was noted early (26) and often confirmed. Closer scrutiny of this fact seems to indicate, however, that this difference is firstly of a quantitative kind, and, secondly, that it is restricted to relatively few organs. It is particularly obvious in the non-pregnant uterus of various animals, but it should be remembered that the uterus occupies quite a special position in being an organ with larger variations in function than perhaps any other organ. The significance of this large difference in effect of the two mediators is still obscure, as is true also for the fowl's rectal caecum. Again in their effects on the digestive tract as a whole, noradrenaline and adrenaline do not differ very greatly. On other smooth muscle organs such as the bronchial muscles, the iris and the bladder, adrenaline is likewise more active than noradrenaline although the difference is moderate.

Two groups of reactions seem to merit particular attention, namely, the circulatory effects and the metabolic effects. Of the two agents noradrenaline is the more powerful vasoconstrictor and since it does not raise the minute volume of the heart while raising the mean blood pressure it must exert a general vasoconstrictor action, as first demonstrated in a careful and extensive study by Goldenberg and associates (126) and later confirmed by several authors (24, 162, 196). This is in contrast to adrenaline which, while increasing the minute volume of the heart (126), does not raise the mean pressure to any marked extent but sometimes even lowers it. This is compatible only with the conclusion that the net peripheral vascular effect of adrenaline is vasodilatation. The possibility remains, however, that in certain vascular areas noradrenaline may cause dilatation and adrenaline constriction. As might be expected, noradrenaline dilates the coronary vessels (60, 111) and, according to Smith and Coxe (226), *l*-noradrenaline has a dilating action on isolated coronary arteries of swine which is 2½ times as strong as that of adrenaline. Also noradrenaline exerts a powerful inotropic action on mammalian heart muscle (123) and a marked positive chronotropic effect on the in a more fundamental way. Thus the scope of adrenergic activity would be enlarged.

Examples of such differentiation of action of chemically near related compounds are at hand both in the internal secretion of the ovary (oestrone and progesterone) and in the suprarenal cortex, where alterations in the hormone molecule impart striking differences in action. The unitarianism of the "adrenaline era" has given place to a dualism in regard to adrenergic nerve mediators, and it is not improbable that further research will reveal a still more complete situation.

Although it was noted as far back as 1906 (35) and repeatedly confirmed that noradrenaline was considerably less toxic than adrenaline (156, 233), only in the last few years have investigations brought to light a number of important differences in the actions of adrenaline and noradrenaline. It would seem fruitful to try to arrange these differences in a system, although it is certain to suffer from incompleteness at this early date and no doubt will require future modifications.

The rather weaker activity of noradrenaline in comparison with adrenaline on a number of smooth muscle organs was noted early (26) and often confirmed. Closer scrutiny of this fact seems to indicate, however, that this difference is firstly of a quantitative kind, and, secondly, that it is restricted to relatively few organs. It is particularly obvious in the non-pregnant uterus of various animals, but it should be remembered that the uterus occupies quite a special position in being an organ with larger variations in function than perhaps any other organ. The significance of this large difference in effect of the two mediators is still obscure, as is true also for the fowl's rectal caecum. Again in their effects on the digestive tract as a whole, noradrenaline and adrenaline do not differ very greatly. On other smooth muscle organs such as the bronchial muscles, the iris and the bladder, adrenaline is likewise more active than noradrenaline although the difference is moderate.

Two groups of reactions seem to merit particular attention, namely, the circulatory effects and the metabolic effects. Of the two agents noradrenaline is the more powerful vasoconstrictor and since it does not raise the minute volume of the heart while raising the mean blood pressure it must exert a general vasoconstrictor action, as first demonstrated in a careful and extensive study by Goldenberg and associates (126) and later confirmed by several authors (24, 162, 196). This is in contrast to adrenaline which, while increasing the minute volume of the heart (126), does not raise the mean pressure to any marked extent but sometimes even lowers it. This is compatible only with the conclusion that the net peripheral vascular effect of adrenaline is vasodilatation. The possibility remains, however, that in certain vascular areas noradrenaline may cause dilatation and adrenaline constriction. As might be expected, noradrenaline dilates the coronary vessels (60, 111) and, according to Smith and Coxe (226), *l*-noradrenaline has a dilating action on isolated coronary arteries of swine which is 2½ times as strong as that of adrenaline. Also noradrenaline exerts a powerful inotropic action on mammalian heart muscle (123) and a marked positive chronotropic effect on the isolated heart (159), which effects should not be confused with the reflex bradycardia produced in man (24). It remains to be seen whether the peculiar local effect of adrenergic transmitter substances in eliciting the sinus reflex is of physiological importance (145, 146).

These results give a valuable indication as to the functional significance of noradrenaline as a mediator of adrenergic nerve fibres to the heart and vessels. The adrenergic vasomotor nerves play a paramount role in the control of the blood pressure, and it seems logical that this activity should be elicited by a substance which does not cause vasodilatation as the sum of its actions but rather by one having a vasoconstrictor action. Likewise there is no obvious reason why the adjustment of blood pressure, when needed, should be accompanied by tachycardia and an increase in the minute volume of the heart, as would be expected if adrenaline served as the mediator.

No less conspicuous are the differences between the action of noradrenaline and adrenaline on various metabolic processes. Adrenaline in small doses raises the oxygen consumption of the organism whereas noradrenaline is much less active in this respect. Thus, doses of noradrenaline which would increase the mean blood pressure by 50 per cent would hardly influence the oxygen consumption in man, whereas adrenaline has a large effect (126). Similar results are reported in animals (234). The hyperglycaemic and glycogenolytic effects of noradrenaline are much less than those of adrenaline (183, 221), and the same is true for the effect on the ascorbic acid content of the suprarenals (199), on the eosinophils (158) and on the blood potassium level (79). Adrenaline is more active than noradrenaline in enhancing the effect of acetylcholine on a perfused sympathetic ganglion (167). Furthermore, the subjective effects of the two catechol amines show quite distinct features. In man adrenaline causes the well-known feeling of anxiety, but this is hardly noticeable after corresponding doses of noradrenaline. Noradrenaline is said to be rather more active as a diuretic than adrenaline (157) in rats. There is little doubt that future biochemical studies will reveal many more important differences, quantitative or qualitative, between the metabolic effects of noradrenaline and adrenaline.

There is reason to emphasize that the similarities as well as the differences in action between noradrenaline and adrenaline merit equal consideration, but that any one-sided view is likely to give an inadequate concept of the peculiar cooperation of these two agents. The fields of action of these two substances complement each other in an interesting way. The "usefulness" of noradrenaline was, in fact, emphasized several years ago as Stehle, Melville and Oldham (228) wrote "arterenol is a more potent substance than adrenaline . . . yet nature would seem to have made the second rate substance when a better one was more readily available".

Further information is required as to the occasions on which adrenaline may serve wholly or predominantly as mediator. Indications in this direction have not been lacking (19, 178, 179, 219, 242) but more complete evidence is necessary in order to form a definite opinion on each of these cases.

isolated heart (159), which effects should not be confused with the reflex bradycardia produced in man (24). It remains to be seen whether the peculiar local effect of adrenergic transmitter substances in eliciting the sinus reflex is of physiological importance (145, 146).

These results give a valuable indication as to the functional significance of noradrenaline as a mediator of adrenergic nerve fibres to the heart and vessels. The adrenergic vasomotor nerves play a paramount role in the control of the blood pressure, and it seems logical that this activity should be elicited by a substance which does not cause vasodilatation as the sum of its actions but rather by one having a vasoconstrictor action. Likewise there is no obvious reason why the adjustment of blood pressure, when needed, should be accompanied by tachycardia and an increase in the minute volume of the heart, as would be expected if adrenaline served as the mediator.

No less conspicuous are the differences between the action of noradrenaline and adrenaline on various metabolic processes. Adrenaline in small doses raises the oxygen consumption of the organism whereas noradrenaline is much less active in this respect. Thus, doses of noradrenaline which would increase the mean blood pressure by 50 per cent would hardly influence the oxygen consumption in man, whereas adrenaline has a large effect (126). Similar results are reported in animals (234). The hyperglycaemic and glycogenolytic effects of noradrenaline are much less than those of adrenaline (183, 221), and the same is true for the effect on the ascorbic acid content of the suprarenals (199), on the eosinophils (158) and on the blood potassium level (79). Adrenaline is more active than noradrenaline in enhancing the effect of acetylcholine on a perfused sympathetic ganglion (167). Furthermore, the subjective effects of the two catechol amines show quite distinct features. In man adrenaline causes the well-known feeling of anxiety, but this is hardly noticeable after corresponding doses of noradrenaline. Noradrenaline is said to be rather more active as a diuretic than adrenaline (157) in rats. There is little doubt that future biochemical studies will reveal many more important differences, quantitative or qualitative, between the metabolic effects of noradrenaline and adrenaline.

There is reason to emphasize that the similarities as well as the differences in action between noradrenaline and adrenaline merit equal consideration, but that any one-sided view is likely to give an inadequate concept of the peculiar cooperation of these two agents. The fields of action of these two substances complement each other in an interesting way. The "usefulness" of noradrenaline was, in fact, emphasized several years ago as Stehle, Melville and Oldham (228) wrote "arterenol is a more potent substance than adrenaline . . . yet nature would seem to have made the second rate substance when a better one was more readily available".

Further information is required as to the occasions on which adrenaline may serve wholly or predominantly as mediator. Indications in this direction have not been lacking (19, 178, 179, 219, 242) but more complete evidence is necessary in order to form a definite opinion on each of these cases.

An attempt has been made in this section to provide an explanation of the fact that noradrenaline appears as the chief adrenergic nerve mediator, assuming that the most important "routine" functions of the adrenergic nerves are connected with the regulation of circulation. In performing the minor adjustments of homeostasis in which adrenergic fibres serve the circulatory system, there is probably no need for concomitant changes in either oxygen consumption or blood sugar, nor in the distribution of the eosinophils or the emotional pattern. All the metabolic effects, so characteristic of adrenaline, point to a supporting mechanism, the stimulation of which would produce results such as were attributed by Cannon to the adrenaline of the suprarenals in its role as an emergency substance. The reason for the occurrence of adrenaline as an adrenergic nerve mediator as well as for its presence in large quantities in the suprarenals, might therefore be sought in its possible local usefulness in certain conditions of stress.

VI. ADRENERGIC NERVE MEDIATORS IN BODY FLUIDS

a) *Aqueous humour*

Sympathomimetic activity has been repeatedly observed in aqueous humour of various animals (10, 95, 181) and it has been noted that it is increased after sitmulation of the cervical sympathetic nerves. It is therefore likely that the activity is derived from the adrenergic nerves of the innervated structures in contact with the aqueous humour. Attempts to analyze the action have shown that the relative amounts of noradrenaline and adrenaline differ in various preparations, but that the relative amount of adrenaline is comparatively high. Addition of strong alkali to concentrated extracts gives a red coloration (98). Although adrenaline is more powerful in causing dilatation of the pupil, noradrenaline also has this effect; hence it is not possible to draw any conclusions concerning the nature of the adrenergic nerve mediators from simple observations of the effects on the pupil.

b) *Exudates*

A few observations have been made on the sympathomimetic activity of peritoneal exudates in thyroidectomized sheep (98) and in scarification fluid (9). In both instances adrenergic substances could be demonstrated in small amounts.

c) *Various secretions*

Noradrenaline has been demonstrated in the seminal fluid of the bull (28). Whether the peculiar occurrence of large amounts of adrenaline in the parotid gland of *Bufo marinus* is due to adrenergic nerve activity is still unsettled (110). After sectioning the nerves to the parotid gland in *Bufo arenarum* the amount of adrenaline is often greatly diminished (110). However, this does not seem to be the case in all species of toad (73). The nature of the sympathomimetic agent

An attempt has been made in this section to provide an explanation of the fact that noradrenaline appears as the chief adrenergic nerve mediator, assuming that the most important "routine" functions of the adrenergic nerves are connected with the regulation of circulation. In performing the minor adjustments of homeostasis in which adrenergic fibres serve the circulatory system, there is probably no need for concomitant changes in either oxygen consumption or blood sugar, nor in the distribution of the eosinophils or the emotional pattern. All the metabolic effects, so characteristic of adrenaline, point to a supporting mechanism, the stimulation of which would produce results such as were attributed by Cannon to the adrenaline of the suprarenals in its role as an emergency substance. The reason for the occurrence of adrenaline as an adrenergic nerve mediator as well as for its presence in large quantities in the suprarenals, might therefore be sought in its possible local usefulness in certain conditions of stress.

VI. ADRENERGIC NERVE MEDIATORS IN BODY FLUIDS

a) *Aqueous humour*

Sympathomimetic activity has been repeatedly observed in aqueous humour of various animals (10, 95, 181) and it has been noted that it is increased after sitmulation of the cervical sympathetic nerves. It is therefore likely that the activity is derived from the adrenergic nerves of the innervated structures in contact with the aqueous humour. Attempts to analyze the action have shown that the relative amounts of noradrenaline and adrenaline differ in various preparations, but that the relative amount of adrenaline is comparatively high. Addition of strong alkali to concentrated extracts gives a red coloration (98). Although adrenaline is more powerful in causing dilatation of the pupil, noradrenaline also has this effect; hence it is not possible to draw any conclusions concerning the nature of the adrenergic nerve mediators from simple observations of the effects on the pupil.

b) *Exudates*

A few observations have been made on the sympathomimetic activity of peritoneal exudates in thyroidectomized sheep (98) and in scarification fluid (9). In both instances adrenergic substances could be demonstrated in small amounts.

c) *Various secretions*

Noradrenaline has been demonstrated in the seminal fluid of the bull (28). Whether the peculiar occurrence of large amounts of adrenaline in the parotid gland of *Bufo marinus* is due to adrenergic nerve activity is still unsettled (110). After sectioning the nerves to the parotid gland in *Bufo arenarum* the amount of adrenaline is often greatly diminished (110). However, this does not seem to be the case in all species of toad (73). The nature of the sympathomimetic agent found in the salivary glands of certain species of *Octopus* (84) and its relation to adrenergic nerve mediators need further elucidation. It should be of interest to study the occurrence of sympathomimetic agents in tears and perspiration and to correlate their actions with those of stimulation of the corresponding adrenergic nerves.

d) *Blood*

There is a vast literature concerning the appearance of sympathomimetic agents in blood; however, in a large number of these studies the methods that have been employed cannot be regarded as adequate. The literature on this subject will not be reviewed here but the reader is referred to a series of papers of Lehmann (171) and others (4, 5, 46, 182, 240) from which further references may be obtained. Since there is every reason to assume that noradrenaline and adrenaline should be present in the blood, deriving from the suprarenal medulla or from the adrenergic nerve mediators (sympathin), as indeed is indicated by their presence in urine, their demonstration presumably depends upon the discovery of sufficiently sensitive methods.

In blood from human arm veins and in blood from the general circulation of animals being slaughtered, small amounts of sympathomimetic activity corresponding to 1–2 μg. of noradrenaline per 100 ml. of blood were found (107). The blood was extracted with acid alcohol and the extract tested on the cat's blood pressure after removal of depressor substances by means of fuller's earth. Since the action was typical of noradrenaline it is inferred that it derived chiefly from adrenergic nerves and not from the suprarenals which contain, in man and cattle, about 75 per cent of adrenaline and presumably secrete adrenaline in a similar proportion. The concentration of noradrenaline found in whole blood probably would exceed that in plasma since it is known that a certain proportion of the catechol amines is taken up by the red corpuscles (22). A noradrenaline-like substance has also been observed in cross-circulation experiments (202).

The appearance of adrenergic nerve mediators in venous blood coming from organs whose adrenergic nerves have been stimulated has partly been dealt with in section IV. Peart (203) succeeded in showing that stimulation of the splenic nerves considerably increased the noradrenaline content of splenic vein blood. On some occasions he also found a small proportion of adrenaline in such blood, an observation which is in accord with the findings that the splenic nerves and the spleen may contain a small amount of adrenaline in addition to noradrenaline. Similar experiments have subsequently been undertaken on other organs (187–189, 241). Table V shows some figures obtained from venous blood on stimulation of various organs.

e) *Urine*

Holtz and coworkers were the first to discover that urine normally contained sympathomimetic substances ("urosympathin") which were considered to consist of a mixture of noradrenaline, adrenaline and hydroxytyramine (152). In found in the salivary glands of certain species of *Octopus* (84) and its relation to adrenergic nerve mediators need further elucidation. It should be of interest to study the occurrence of sympathomimetic agents in tears and perspiration and to correlate their actions with those of stimulation of the corresponding adrenergic nerves.

d) Blood

There is a vast literature concerning the appearance of sympathomimetic agents in blood; however, in a large number of these studies the methods that have been employed cannot be regarded as adequate. The literature on this subject will not be reviewed here but the reader is referred to a series of papers of Lehmann (171) and others (4, 5, 46, 182, 240) from which further references may be obtained. Since there is every reason to assume that noradrenaline and adrenaline should be present in the blood, deriving from the suprarenal medulla or from the adrenergic nerve mediators (sympathin), as indeed is indicated by their presence in urine, their demonstration presumably depends upon the discovery of sufficiently sensitive methods.

In blood from human arm veins and in blood from the general circulation of animals being slaughtered, small amounts of sympathomimetic activity corresponding to 1–2 μg. of noradrenaline per 100 ml. of blood were found (107). The blood was extracted with acid alcohol and the extract tested on the cat's blood pressure after removal of depressor substances by means of fuller's earth. Since the action was typical of noradrenaline it is inferred that it derived chiefly from adrenergic nerves and not from the suprarenals which contain, in man and cattle, about 75 per cent of adrenaline and presumably secrete adrenaline in a similar proportion. The concentration of noradrenaline found in whole blood probably would exceed that in plasma since it is known that a certain proportion of the catechol amines is taken up by the red corpuscles (22). A noradrenaline-like substance has also been observed in cross-circulation experiments (202).

The appearance of adrenergic nerve mediators in venous blood coming from organs whose adrenergic nerves have been stimulated has partly been dealt with in section IV. Peart (203) succeeded in showing that stimulation of the splenic nerves considerably increased the noradrenaline content of splenic vein blood. On some occasions he also found a small proportion of adrenaline in such blood, an observation which is in accord with the findings that the splenic nerves and the spleen may contain a small amount of adrenaline in addition to noradrenaline. Similar experiments have subsequently been undertaken on other organs (187–189, 241). Table V shows some figures obtained from venous blood on stimulation of various organs.

e) Urine

Holtz and coworkers were the first to discover that urine normally contained sympathomimetic substances ("urosympathin") which were considered to consist of a mixture of noradrenaline, adrenaline and hydroxytyramine (152). In subsequent papers members of Holtz's research group have expressed the opinion that the physiological effects observed are due chiefly or entirely to noradrenaline in man and some animals (154, 168). Recent investigations (101) have shown, however, that hydroxytyramine is the chief catechol amine in normal human urine. A large part of the catechol amines appear in free form (101).

The assumption that noradrenaline and adrenaline in urine derive chiefly from the adrenergic nerve mediators and not from the suprarenals is supported by the fact that the relative amounts of the two agents in urine is about the same as their relative amounts in adrenergic nerve tissue, noradrenaline appearing in 3–5 times as high concentration as adrenaline (102). The preponderance of noradrenaline accords with the findings in blood and in organs and nerves generally. Only a small fraction of the adrenergic nerve mediators present in the blood appears in urine, however, judging from experiments (106) in which noradrenaline was continuously infused in man at a fixed rate and the amounts excreted estimated. Thus out of 1 mg. *dl*-noradrenaline, infused during 1 hour, only 12 μg. *l*-noradrenaline in excess of the resting secretion was recovered in urine, or 2.4 per cent of the dose.

TABLE V

VENOUS BLOOD	NORADRENALINE	ADRENALINE	REFERENCE
	μg./ml.	*μg./ml.*	
Cat splenic	0.05–0.5	0–0.01	(203)
" "	0.13	traces occasionally	(189)
Rabbit uterine	0.046	traces occasionally	(187)
Cat hepatic	0.48	traces occasionally	(189)
Cat uterine	0.037	0.005	(188)

The significance of the comparatively large amounts of hydroxytyramine appearing in urine is as yet obscure. There are reasons for believing that this substance is formed by decarboxylation of DOPA since the decarboxylase of this amino acid occurs in a variety of organs. So far there are no indications either that hydroxytyramine is liberated on adrenergic nerve stimulation or that it serves as an adrenergic mediator, but admittedly there would be great difficulties in detecting this substance in the presence of noradrenaline and adrenaline by the methods available at present.

When discussing the significance of the appearance of adrenaline and noradrenaline in the urine, the secretion from the suprarenals and other chromaffine cell groups should be considered. It should be possible, however, to determine the extra-adrenal production by estimating the excretion in the urine after demedullation of the suprarenals, knowing the relationship between administered noradrenaline and adrenaline and the output of these substances in urine.

The increased output of noradrenaline in urine during heavy muscular work (103) is in all probability a sign of increased adrenergic nerve activity during this activity.

VII. EFFECT OF DEGENERATION AND REGENERATION OF THE ADRENERGIC NERVES ON THE OCCURRENCE OF THE MEDIATORS IN ORGANS

The following evidence lends strong support to the hypothesis that the sympathomimetic agents present in an extract of an organ are derived from the adrenergic nerves found in that organ: extracts of the nerve-free placenta are lacking in sympathomimetic activity (87, 219); likewise, this activity is almost entirely absent from organs in which degeneration of the adrenergic fibers has occurred as a result of section of the nerves (65, 98, 130). An interesting fact about the distribution of adrenaline and noradrenaline has been observed in extracts of organs after denervation. In the spleen of sheep the amounts per gram of fresh tissue are about 3 μg. of *l*-noradrenaline and 0.1–0.2 μg. of adrenaline, respectively; but after denervation the relative adrenaline content has been as high as 50 per cent of the small catechol amine residue (98). Similar observations have been made on the submaxillary gland of sheep, the adrenaline percentage showing an increase from about 10 or 20 to around 50 per cent. This may not mean a shift in the distribution of the mediators of the few remaining adrenergic nerves, but may equally well, or better, be explained by the presence of chromaffine cells in the organs. In fact the difference in the total adrenaline content of the submaxillary gland of the sheep in normal and denervated glands may be so small so as to cast serious doubt on the view that the adrenaline found in the normally innervated gland actually represents an adrenergic nerve mediator. The possibility must be considered, therefore, that adrenaline in an organ may partially represent the production of chromaffine cells which would not degenerate on denervation. On the other hand the decrease in the apparent adrenaline content of the heart after denervation approximately parallels the fall in noradrenaline (130). [For distribution of sympathetic fibres consult (47)].

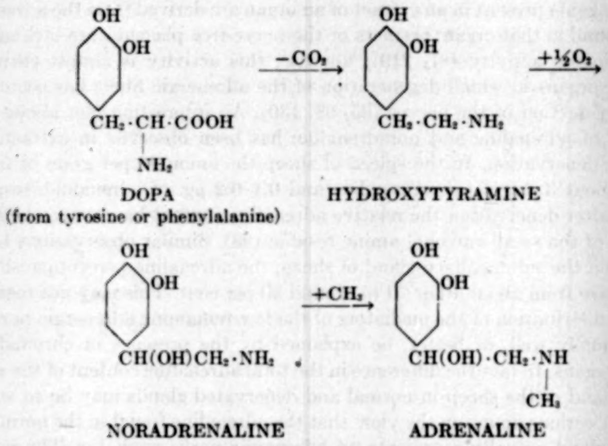
Of considerable importance are the experiments of Goodall, which have shown that after a certain lapse of time sympathomimetic substances reappear in previously denervated organs (130). After removing the sympathetics in the neck and the stellate ganglia in the sheep he found that the noradrenaline and adrenaline content of extracts of the heart fell nearly to zero. About 8 weeks after denervation, these extracts began to show an increase in their content of these substances and after 10–12 weeks the content was about normal. At this time the regeneration of adrenergic postganglionic fibres growing out from the sympathetic chain has proceeded enough to replenish the stores of adrenergic nerve mediators in the organ. No consistent alteration of the relative amounts of noradrenaline and adrenaline was noticed after regeneration.

VIII. FORMATION OF ADRENERGIC NERVE MEDIATORS

a) Chemical

Many theories have been proposed in the past for the formation of adrenaline *in vivo*. The most important contributions to what now seems to be the probable mode of formation have come from Holtz, Heise and Lüdtke (153) who discovered

DOPA-decarboxylase, and from Blaschko (38) who proposed a reaction series including noradrenaline. The biosynthesis may tentatively be assumed to take the following course:



This sequence of reactions is supported by a fair amount of evidence, although circumstantial in some points. If the first step is a decarboxylation of DOPA this substrate, as well as the enzyme, should be present in adrenergic neurons or nerve endings. Thus far DOPA has been demonstrated only in suprarenal extracts from thyroidectomized sheep (129). Hydroxytyramine is also found in such extracts as well as in heart extract (128) and in extracts of urine (101, 102). How the hydroxyl group is introduced into the side-chain of hydroxytyramine is unknown. N-methylation of noradrenaline to form adrenaline has been demonstrated in the suprarenal (56). The methyl group is supplied by methionine (163).

The possibility of adrenaline formation from various substances has been the subject of several studies (80) including some in which isotopes have been used (139), but none will be treated in detail here.

The possibility of the formation of noradrenaline by decarboxylation of the corresponding amino acid, dihydroxyphenylserine, has been considered, and small amounts of noradrenaline have been found on incubating the amino acid with enzyme extracts from guinea pig kidney (33, 41). Feeding rabbits with dihydroxyphenylserine seems to increase the urinary noradrenaline (220).

b) *Local*

Some information as to the site of formation of the adrenergic nerve mediators may be gained by a comparison of their amounts in the nerve and the corresponding organ. Such a comparison shows, for the splenic nerves and the spleen of the cow, that the content is some 5 times higher in the nerves than in the organ. If the noradrenaline and adrenaline are confined to the nervous structures this would mean that the amounts per unit weight of nerve are many times higher in the peripheral parts (the nerve endings) than in the trunk. Attempts have been made to explain this by the assumption that the whole of the neurone contains the enzyme system necessary for the formation of the mediators but that the optimal conditions with regard to substrate supply, co-enzymes and ionic environment are found only in the nerve end regions (97).

The further possibility has been considered that the mediators should be formed in the central parts of the neurone and carried peripherally by the axoplasm flow (208, 232). This is rather unlikely for the reason that the axoplasm flow is very slow and the concentration in the nerve trunk low. Prolonged stimulation of sympathetic fibres does not diminish their content of mediator substances (180).

c) *Storage*

It has to be assumed that the concentration of adrenergic nerve mediators is relatively high in the terminal nerve structures (see p. 252). Assuming that the proportion of what may be counted as nerve endings in the spleen is 0.2 per cent of the weight of the organ in the cow, an estimate which is probably on the high side, the total amount of catechols being about 4 µg. per gram of organ, this would correspond to 2000 µg. per gram in the terminal structures. This calculation is admittedly somewhat arbitrary, but probably is of the right order of magnitude. Such a figure, however, would fall well within the range of the amounts found in the suprarenal medulla and shows that, basically, storage facilities of this order are conceivable. In fact, chromaffine cell tissue may contain as much as 10 mg. per gram and even more.

The relatively high concentration of mediators in the terminal structures indicates some special storage mechanism. This is not known, but attention should be drawn to the fact that phospholipids have the properties of combining with adrenaline and noradrenaline (25, 87, 164, 166, 201). The resulting product is soluble in ether or chlorofrom, solvents in which pure adrenaline or noradrenaline are practically insoluble. At a neutral or slightly alkaline reaction the proportion bound is considerable but diminishes rapidly at acid reaction (201). On the basis of such a linkage of the mediator substances to phospholipids a mechanism for their liberation has been tentatively suggested. Also it has been found that adrenaline may be stored in the red blood corpuscles (22).

d) *Estimation of adrenergic nerve mediator production*

If the normal urinary output of noradrenaline is 12 µg. per hour (102) the amount of noradrenaline leaking into the blood would be of the order of 40–80 µg. per hour if the same relation between administered and excreted noradrenaline holds as in the experiments quoted on p. 264 (106). The production of *l*-noradrenaline appearing in the blood in a resting state thus would be limited to 0.011–0.022 µg. per kg. per minute, assuming a body weight of 60 kg.

This figure is not incompatible with the findings of Goldenberg and associates (126) regarding the action of continuous infusion of small amounts of noradrenaline in man, where 0.05 μg. *l*-noradrenaline per minute gave a barely perceptible pressure rise in normotensives.

e) Differential activation of noradrenaline and adrenaline producing fibres

The hypothesis has been advanced that two pharmacologically distinct types of cells exist in the sympathetic ganglia (224). According to this hypothesis one kind ('D'cells) produce sympathin I and the 'C' cells sympathin E, these terms designating adrenaline and noradrenaline, respectively. There is perhaps some support for this concept in the results of Brauner, Brücke and Kaindl (50) who noticed certain differences in the kind of response to sympathetic stimulation caused by occlusion of the carotids and by direct hypothalamic stimulation. In the latter case the mediator seemed to be predominantly adrenaline whereas in reflex liberation noradrenaline predominated. Their findings are of interest in suggesting that centrally-induced adrenergic nerve activity is connected with the liberation of adrenaline in a higher degree whereas the "routine" vascular reflexes make use of noradrenaline as the transmitter. Further study in this interesting field is highly desirable.

f) Oxidation products of adrenaline in relation to adrenergic nerve mediators

Alterations of the pharmacodynamic action of adrenaline at various stages of oxidation have suggested that the products formed may be the cause of the phenomena attributed to the postulated sympathins I and E (21, 143). There is, however, little experimental evidence to support the interesting hypothesis that such products as adrenoxin may serve as adrenergic nerve mediators, although it is possible that they occur and take part in the effects observed. It has also been suggested that adrenochrome (134), the red oxidation product of adrenaline, might serve as a precursor in the formation of adrenaline at the nerve endings (18), but it has never been demonstrated that the indole ring of this compound can be opened. (For further discussion of the metabolism of hydroxyphenol derivatives, consult 43, 44, 141, 172, 191, 215).

IX. INACTIVATION AND EXCRETION

A few facts about the inactivation and excretion of the adrenergic nerve mediators will be briefly mentioned here. Though easily oxidized by molecular oxygen at neutral or alkaline reaction in pure solutions, noradrenaline and adrenaline show a remarkable stability in organs or body fluids or extracts thereof. As preserving factors certain amino acids (1), ascorbic acid and other reducing substances come into account. The preservation in blood is very effective as shown by the fact that heparinized suprarenal vein blood was found to have the same activity after several days in the refrigerator at +5° C. as immediately after sampling (121). Also in urine the inactivation is slight even after 48 hours at room temperature (102). Adrenaline and noradrenaline resist heating in normal acid for a considerable length of time, although the biological activity of the laevo-isomers is reduced 50 per cent by heating in N/10 solution for about 1 hour, owing to racemization (102).

The adrenergic nerve mediators are removed by enzymic inactivation and by elimination through the kidneys. Direct oxidation with oxygen seems to be of less importance. Amine oxidase is present in a variety of organs and tissues and inactivates both noradrenaline and adrenaline (39). An interesting fact has been observed by Blaschko and Burn (40) who found that noradrenaline is more readily broken down than adrenaline by amine oxidase from rabbit liver. The same is true for the enzyme present in the splenic nerves of cattle (237). Whether catechol oxidase also functions in inactivating the nerve mediators has not been demonstrated with certainty but such seems possible in view of the red coloration of solutions of adrenaline and noradrenaline on incubation with nervous tissue or spleen (237).

The inactivation of liberated adrenergic nerve transmitters seems to take place largely in the kidney (13). An interesting hypothesis with regard to the inactivation has been offered (60) on the basis of observations of wide variations in the relative activity of adrenaline and noradrenaline on the denervated nictitating membrane of the cat. The effects may be explained by assuming that some inactivating factor is removed by denervation which thus causes hypersensitivity (69; cf. 175).

Part of the adrenergic nerve mediators are inactivated by conjugation and excreted in the urine as ethereal sulfates (34, 210, 211) or as glucuronides (74, 81). Finally a certain proportion is excreted in the free form (102, 168).

The inactivation rate in the body could be determined if the amounts liberated into the blood and the blood concentration were known. The inflow rate might be approximately calculated from the urinary output, correlating known inflows, for example by infusion, with the output in urine which can be measured. The estimation of the blood concentration during infusion of noradrenaline or adrenaline requires sensitive methods.

X. ADRENERGIC NERVE MEDIATORS IN METABOLIC DISORDERS

It is likely that the enzymic formation of adrenergic nerve mediators are subject to alterations in quantity and in the relative amounts of their constituents during various abnormal conditions. Several observations seem to indicate that, for instance, during thyroid hyperactivity there is an increased formation of the adrenergic mediators. The experimental data obtained by direct estimation are very few as yet. It has been reported, however, that after thyroidectomy as well as following thyroxine administration in the sheep the catechol amines of the heart are increased 20 to 50% and that the relative amount of adrenaline is decreased (130). In cats and rats large changes may occur in the catechol amine content of the heart during various conditions (207).

Hypophysectomy does not seem to alter the catechol amine content in organs, but a shift towards a larger proportion of adrenaline has been noted (104). In vitamin B_1-deficiency the noradrenaline content of the heart of the rat is increased (130, 207). More refined methods will probably be required to ascertain whether the mediators liberated into the blood stream will show any modifications in amount and composition in endocrinological and metabolic disorders.

A striking alteration in the proportions of noradrenaline and adrenaline in urine has been noted as a result of ACTH and cortisone treatment of human patients (105). Soon after beginning the treatment the noradrenaline content of the urine decreased markedly, leaving the adrenaline almost unchanged. On some occasions only adrenaline was found in the urine. Whether this change is due to a difference in the composition of the mediators or is due to alterations in the inactivation process is not known.

XI. ADRENERGIC NERVE MEDIATORS IN INVERTEBRATES

This subject is extensively dealt with in an excellent review by Frédéricq (114). He points out that adrenergic fibres probably exist in the cardioaccelerator nerves of the *Gastropod Aplysia* and in *Cephalopods*. There is some evidence that the cardio-moderator nerves of *Cephalopods* are tyraminergic. Whether noradrenaline serves as a mediator in invertebrates is not clear, although it seems possible judging from experiments on *Annelids*, where extracts of muscle have shown some characteristics of noradrenaline (95). In extracts of the posterior salivary glands of *Octopods* an active sympathomimetic agent has been described which might be tyramine (144) or methyltyramine (84).

XII. ADRENERGIC NERVE MEDIATOR SUBSTANCES IN THERAPEUTIC USE

Adrenaline has found therapeutic use in the treatment of hypotension, in surgical shock and as a means of stimulating heart activity in circulatory collapse. After it had been established that noradrenaline is the chief physiological vasoconstrictor agent, interest turned in the direction of using this substance instead of adrenaline. Encouraging results have already been reported by Goldenberg and his associates (124) who were the first to put noradrenaline to clinical test. They found noradrenaline very useful, and superior to adrenaline, in restoring the low blood pressure after haemorrhage, in surgical shock and in other conditions. Similar good results, especially in connection with the administration of blood or blood substitutes, have also been reported by other authors (216). Noradrenaline should have many advantages over adrenaline as a blood pressure-restoring substance since it produces pure vasoconstriction without raising the minute volume of the heart, does not increase the oxygen need, does not cause tachycardia but bradycardia, has no untoward psychic effects and is but slightly toxic.

Especially on removal of phaeochromocytomas, whereupon the organism is suddenly deprived of a large inflow of noradrenaline, a continuous administration of this substance should be of great use, as indeed has proved to be the case.

XIII. SUMMARY

The chief points in the present concept of the adrenergic nerve mediators may be briefly summarized as follows:

1. Convincing evidence has been produced to show that *l*-noradrenaline is the chief adrenergic nerve mediator in mammals. In addition *l*-adrenaline occurs admixed in various small amounts with the noradrenaline. The hypothesis of purely

excitatory or purely inhibitory sympathins is no longer tenable. The liberation of other related substances such as hydroxytyramine, tyramine and oxidation products of dihydroxyphenyl derivatives in adrenergic nerve activity has not been proven in mammals.

2. The adrenergic nerve mediators *l*-noradrenaline and *l*-adrenaline can account for most actions observed on stimulation of adrenergic nerves.

3. Noradrenaline and adrenaline are formed in the adrenergic neurons and appear in small quantities in the nerve trunks and in larger amounts in the innervated organs, presumably occurring in the terminal nerve structures.

4. The adrenergic nerve mediators disappear from an organ after degeneration of the nerves but reappear on nerve regeneration.

5. There is some evidence in favour of the formation of noradrenaline by decarboxylation of dihydroxyphenylalanine and subsequent introduction of a hydroxyl group in the side chain of the hydroxytyramine so formed.

6. The adrenergic nerve mediators are rapidly inactivated in the tissues by enzymes (amine oxydase and catechol oxidase). A smaller part is excreted in free or conjugated form in the urine.

7. The physiological actions of noradrenaline and adrenaline differ in many important respects. The main function of noradrenaline seems to be the normal control of the circulation, while adrenaline produces various effects in conditions of stress and emergency.

REFERENCES

1. Abderhalden, E. and Gellhorn, E. Das Verhalten des Herzstreifenpräparates (nach Loewe) unter verschiedenen Bedingungen. III. Mitteilung. Weitere Versuche zur Kenntnis der Adrenalinwirkung. Pflügers Archiv **199**: 437–456, 1923.
2. Ahlquist, R. P. A study of the adrenotropic receptors. Am. J. Physiol., **153**: 586–600, 1948.
3. Allen, W. J., Barcroft, H. and Edholm, O. G. Action of adrenaline on blood vessels in human skeletal muscle. J. Physiol., **105**: 255–267, 1946.
4. Alles, G. A. The comparative physiological actions of dl-β-phenylisopropylamines; pressor effect and toxicity. J. Pharmacol. & Exper. Therap., **47**: 339–354, 1933.
5. Annersten, S., Grönwall, A. and Köiw, E. The fluorimetric determination of adrenaline in blood plasma. Scandinav. J. Clin. & Lab. Investigation., **1**: 60–69, 1949.
6. Åström, A. Anti-sympathetic action of sympathomimetic amines. Acta physiol. Scandinav., **18**: 295–307, 1949.
7. Auerbach, M. E. and Angell, E. The determination of arterenol in epinephrine. Science, **109**: 537–538, 1949.
8. Babsky, E. B. Sur la formation de substances physiologiquement actives dans les fibres nerveux. Bull. Biol. Med. expér. URSS, **5**: 51–54, 1938.
9. Bacq, Z. M. Action de la sympathine sur l'iris. Compt. rend. Soc. de biol., **112**: 1551–1552, 1933.
10. Bacq, Z. M. Recherches sur la physiologie du système nerveux autonome. III. Les proprietés biologiques et physico-chimiques de la sympathine comparées à celles de l'adrénaline. Arch. internat. de physiol., **36**: 167–246, 1933.
11. Bacq, Z. M. La pharmacologie du système nerveux autonome et particulièrement du sympatique, d'après la theorie neurohumorale. Ann. Physiol., **10**: 467–528, 1934.
12. Bacq, Z. M. La transmission chimique des influx dans le système nerveux autonome. Ergebn. d. Physiol., **37**; 82–185, 1935.
13. Bacq, Z. M. Recherches sur la physiologie et la pharmacologie du système nerveux autonome; role du foie et des viscères abdominaux dans la déstruction de l'adrénaline. Arch. internat. de Physiol., **45**: 1–5, 1937.
14. Bacq, Z. M. La substance sympathicomimétique des extraits de rate. Compt. rend. Soc. de biol.. **141**: 537–538, 1947.
15. Bacq, Z. M. Artérénol ou adrénaline dans les extraits de tissus. Compt. rend. Soc. de biol., **141**: 963–964, 1947.
16. Bacq, Z. M. L'acétylcholine et l'adrénaline chez les invertébrés. Biol. Rev. Cambridge Phil. Soc., **22**: 73–91, 1947.
17. Bacq, Z. M. Facts and theories about sympathins. Science, **108**: 135, 1948.
18. Bacq, Z. M. Métabolisme et synthèse de l'adrénaline. Bull. Acad. Suisse Sci. Méd., **4**: 127–140, 1948.
19. Bacq, Z. M. and Fischer, P. Nature de la substance sympathicomimétique extraite des nerfs ou des tissus des mammifères. Arch. internat. de Physiol., **55**: 73–91, 1947.

20. Bacq, Z. M. and Frédéricq, H. Action de la sympathine splénique sur la membrane nictitante du chat. Sensibilisation par la cocaine, désensibilisation par le 933 F. Arch. internat. de Physiol., **41:** 334–339, 1935.

21. Bacq, Z. M. and Heirman, P. Recherches sur l'adrénoxine. VI. Discussion générale des faits acquis. Arch. internat. de Physiol., **50:** 153–168, 1940.

22. Bain, W. A., Gaunt, W. E. and Suffolk, S. F. Observations on the inactivation of adrenaline by blood and tissues in vitro. J. Physiol., **91:** 233–253, 1937.

23. Barclay, J. A., Cooke, W. T. and Kenney, R. A. Observations on the effects of adrenaline on renal function and circulation in man. Am. J. Physiol., **151:** 621–625, 1947.

24. Barcroft, H. and Konzett, H. On the actions of noradrenaline, adrenaline and isopropyl noradrenaline on the arterial blood pressure, heart rate and muscle blood flow in man. J. Physiol., **110:** 194–204, 1949.

25. Barger, G. Some applications of organic chemistry to biology and medicine. Baker Lecture, **5:** McGraw-Hill, New York, 1930.

26. Barger, G. and Dale, H. H. Chemical structure and sympathomimetic action of amines. J. Physiol., **41:** 19–59, 1910–1911.

27. Barron, D. H. Fortgesetzte Untersuchungen über Wärmebildung in normalen und sympathikuslosen Muskeln. Ztschr. f. Biol., **95:** 575–587, 1934.

28. Beauvallet, M. and Brochart, M. Présence de nor-adrénaline dans le sperme du Taureau. Compt. rend. Soc. de biol., **3-4:** 237–239, 1949.

29. Benitez, D., Herreros, M., Middleton, S. and Talesnik, J. Influencia de la adrenalina sobre la secreción de la glandula submaxilar inducida por la acetilcolina y estimulación de la cuerda del timpano. Boll. Soc. Biol. Santiago de Chile, **6/2:** 86, 1949.

30. Bergström, S., Euler, U. S. v. and Hamberg, U. Isolation of noradrenaline from suprarenal medulla. Acta physiol. Scandinav., **20:** 101–108, 1950.

31. Bergström, S. and Sjövall, J. The separation of adrenaline and noradrenaline by partition chromatography on a preparative scale. Acta physiol. Scandinav., in press.

32. Beyer, K. H. Sympathomimetic amines; relation of structure to their action and inactivation. Physiol. Rev., **26:** 169–197, 1946.

33. Beyer, K. H., Blaschko, H., Burn, J. H. and Langemann, H. Enzymic formation of nor-adrenaline in mammalian tissue extracts. Nature **165:** 926, 1950.

34. Beyer, K. H. and Shapiro, S. H. The excretion of conjugated epinephrine related compounds. Am. J. Physiol., **144:** 321–330, 1945.

35. Biberfeld, J. Pharmakologische Eigenschaften eines synthetisch dargestellten Suprarenins und einiger seiner Derivate. Med. Klin., 1177–1179, 1906.

36. Binet, L. and Burstein, M. Sur la vasodilatation périphérique d'origine adrénalinique. Compt. rend. Soc. de biol., **141:** 630–633, 1947.

37. Bing, R. J. The formation of hydroxytyramine by extracts of renal cortex and by perfused kidneys. Am. J. Physiol., **132:** 497–503, 1940.

38. Blaschko, H. The activity of 1(-)-dopa decarboxylase. J. Physiol., **101:** 337–349, 1942.

39. Blaschko, H. Substrate specificity of amino-acid decarboxylases. Biochimica et Biophysica Acta, **4:** 130–137, 1950.

40. Blaschko, H. and Burn, J. H. Oxidation of adrenaline and noradrenaline by amine oxidase. J. Physiol., **112:** 37P, 1951.

41. Blaschko, H., Burn, J. H. and Langemann, H. The formation of noradrenaline from dihydroxyphenylserine. Brit. J. Pharmacol., **5:** 431–437, 1950.

42. Blaschko, H. and Holton, P. Enzymic formation of orthotyramine (o-hydroxyphenylethylamine). J. Physiol., **110:** 482–487, 1949.

43. Blaschko, H., Holton, P. and Stanley, G. H. S. Enzymic formation of pressor amines. J. Physiol., **108:** 427–439, 1949.

44. Blaschko, H., Richter, D. and Schlossmann, H. Oxidation of adrenaline and other amines. Biochem. J., **31:** 2187–2196, 1937.

45. Bloch, W. Zur quantitativen Bestimmung des Adrenalins im Blut mit der Fluoreszenzreaktion. Helv. Physiol. Acta, **6:** 122–129, 1948.

46. Bloor, W. R. and Bullen, S. S. The determination of adrenaline in blood. J. Biol. Chem., **138:** 727–739, 1941.

47. Boeke, J. Die periphere Endausbreitung des sympathischen Systems. Nova Acta Leopoldina, **2:** 209–257, 1935.

48. Bouckaert. J. J. and Jourdan, F. Recherches sur la physiologie et la pharmacodynamie des vaisseaux cérebraux. Arch. internat. de pharmacodyn. et de thérap., **54:** 109–128, 1936.

49. Bovet, D. and Bovet-Nitti, E. Structure et activité pharmacodynamique des médicaments du système nerveux végétatif.—Adrénaline, acéthylcholine, histamine, et leurs antagonistes. S. Karger, Basel, 1948.

50. Brauner, F., Brücke, F. and Kaindl, F. Der Unterschied zwischen direkter und reflektorischer zentraler Sympathicuserregung auf Blutdruck, Milz- und Darmvolumen. Arch. internat. de pharmacodyn. et de thérap., **81:** 357–368, 1950.

51. Brauner, F., Brücke, F. and Kaindl, F. Ueber die Wirkung direkter und reflektorischer Sympathicuserregung bei Beatmung mit Gasgemischen von verschiedenem Sauerstoffgehalt. Arch. internat. de pharmacodyn. et de thérap., **82:** 192–205, 1950.

52. Brooks, Ch.M. Reflex activation of the sympathetic system in the spinal cat. Am. J. Physiol., **106:** 251–266, 1933.

53. Brown, G. L., Goffart, M. and Vianna Dias, M. The effects of adrenaline and of sympathetic stimulation on the demarcation potential of mammalian skeletal muscle. J. Physiol., **3:** 184–194, 1950.

54. Brun, G. C. Mechanism of the variations in the diameter of the abdominal arteries after intravenous injection of adrenaline. Acta pharmacol. et toxicol., **2:** 22–50, 1946.

55. Bülbring, E. The action of adrenaline on transmission in the superior cervical ganglion. J. Physiol., **103**: 55–67, 1944.

56. Bülbring, E. The methylation of noradrenaline by minced suprarenal tissue. Brit. J. Pharmacol., **4**: 234–244, 1949.

57. Burn, J. H. The cardio-vascular action of tyramine. Quart. J. Pharm. & Pharmacol., **3**: 187–204, 1930.

58. Burn, J. H. The action of tyramine and ephedrine. J. Pharmacol. & Exper. Therap., **46**: 75–95, 1932.

59. Burn, J. H. The relation of adrenaline to acetylcholine in the nervous system. Physiol. Rev., **25**: 377–394, 1945.

60. Burn, J. H. and Hutcheon, D. E. The action of noradrenaline. Brit. J. Pharmacol., **4**: 373–380, 1949.

61. Burn, J. H., Hutcheon, D. E. and Parker, R. H. O. Estimation of adrenaline-noradrenaline mixture. Brit. J. Pharmacol., **5**: 142–146, 1950.

62. Burn, J. H. and Tainter, M. L. An analysis of the effect of cocaine on the actions of adrenaline and tyramine. J. Physiol., **71**: 169–193, 1931.

63. Calabro, Q. Riv. Biol., **15**: 99, 1933. Quoted by Babsky.

64. Cannon, W. B. and Bacq, Z. M. Studies on the conditions of activity in endocrine organs. XXVI. A hormone produced by sympathetic action on smooth muscle. Am. J. Physiol., **96**: 392–412, 1931.

65. Cannon, W. B. and Lissák, K. Evidence for adrenaline in adrenergic neurones. Am. J. Physiol., **125**: 765–777, 1939.

66. Cannon, W. B. and Rosenblueth, A. Sympathin E and sympathin I. Am. J. Physiol., **104**: 557–574, 1933.

67. Cannon, W. B. and Rosenblueth, A. A comparison of the effects of sympathin and adrenine on the iris. Am. J. Physiol., **113**: 251–258, 1935.

68. Cannon, W. B. and Rosenblueth. A. Autonomic neuro-effector systems. Macmillan, New York 1937.

69. Cannon, W. B. and Rosenblueth, A. The supersensitivity of denervated structures. A law of denervation. The Macmillan Company, New York, 1949.

70. Cannon, W. B. and Uridil, J. E. Studies on the conditions of activity in endocrine glands. VIII. Some effects on the denervated heart of stimulating the nerves of the liver. Am. J. Physiol., **58**: 353–354, 1921–1922.

71. Chakrabarty, M. L. Double action of adrenaline on the small intestine. Am. J. Physiol., **159**: 457–460, 1949.

72. Chauchard, P. Nouvelles recherches chronaximétriques sur les neurones ganglionnaires sympathiques. Arch. internat. de Physiol., **55**: 37–50, 1947.

73. Chen, R. and Chen, A. Studies on toad poisons. Arch. internat. de pharmacodyn. et de thérap., **47**: 297–317, 1934.

74. Clark, W. G., Akawie, R. I., Pogrund, R. S. and Geissman, T. A. Conjugation of l-epinephrine in vivo. Abstracts of Communications of the XVIII International Physiological Congress, Copenhagen, 1950.

75. Collip, J. B. A non-specific pressor principle derived from a variety of tissues. J. Physiol., **66**: 416–430, 1928.

76. Dale, H. H. On the action of ergotoxine; with special reference to the existence of sympathetic vasodilators. J. Physiol., **46**: 291–300, 1913.

77. Dale, H. H. Nomenclature of fibres in the autonomic system and their effects. J. Physiol., **80**: 10–11, 1934.

78. Dale, H. H. Reizübertragung durch chemische Mittel im peripheren Nervensystem. Sammlung der von der Nothnagel-Stiftung veranstalteten Vorträge 1935, Heft 4.

79. D'Silva, J. L. Action of adrenaline-like substances on the serum potassium. J. Physiol., **108**: 218–225, 1949.

80. Devine, J. Observations on the in vitro synthesis of adrenaline under physiological conditions. Biochem. J., **34**: 21–31, 1940.

81. Dodgson, K. S. and Williams, R. T. Studies in detoxication. 27. The orientation of conjugation in the metabolites of 4-chlorocatechol and 4-chlororesorcinol, with some observations on the fate of (+)-adrenaline, protocatechuic acid and protocatechuic aldehyde in the rabbit. Biochem. J., **45**: 381–386, 1949.

82. Duff, R. S. and Swan, H. J. C. Effects of adrenaline on muscle blood flow. J. Physiol., **112**: 41P, 1951.

83. Engel, A. and Euler, U. S. v. Diagnostic value of increased urinary output of noradrenaline and adrenaline in phaeochromocytoma. Lancet, **259**: 387, 1950.

84. Erspamer, V. Active substances in the posterior salivary glands of octopoda. II. Tyramine and octopamine (oxyoctopamine). Acta pharmacol. et toxicol., **4**: 224–247, 1948.

85. Euler, U. S. v. An adrenaline-like action in extracts from the prostatic and related glands. J. Physiol., **81**: 102–112, 1934.

86. Euler, U. S. v. The presence of a substance with sympathin E properties in spleen extracts. Acta physiol. Scandinav., **11**: 168–186, 1946.

87. Euler, U. S. v. A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relation to adrenaline and noradrenaline. Acta physiol. Scandinav., **12**: 73–97, 1946.

88. Euler, U. S. v. The presence of a sympathomimetic substance in extracts of mammalian heart. J. Physiol., **105**: 38–44, 1946.

89. Euler, U. S. v. Identification of the sympathomimetic ergone in adrenergic nerves of cattle (sympathin N) with laevo-noradrenaline. Acta physiol. Scandinav., **16**: 63–74, 1948.

90. Euler, U. S. v. Preparation, purification and evaluation of noradrenaline and adrenaline in organ extracts. Arch. internat. de pharmacodyn. et de thérap., **77**: 477–485, 1948.

91. Euler, U. S. v. Sympathin, histamine and acetylcholine in mammalian nerves. J. Physiol., **107**: 10-11P, 1948.

92. Euler, U. S. v. Sympathin E and noradrenaline. Science, **107**: 422, 1948.

93. Euler, U. S. v. Histamine as a specific constituent of certain autonomic nerve fibres. Acta physiol. Scandinav., **19**: 85–93, 1949.

94. Euler, U. S. v. The distribution of sympathin N and sympathin A in spleen and splenic nerves of cattle. Acta physiol. Scandinav., **19**: 207–214, 1949.

95. Euler, U. S. v. Noradrenaline (arterenol), adrenal medullary hormone and chemical transmitter of adrenergic nerves. Ergebn. d. Physiol., **46**: 261–307, 1950.

96. Euler, U. S. v. Estimation of adrenaline and nor-adrenaline in tissue extracts. Methods in Medical Research, Year Book Publishers, Chicago, **3**: 131–141, 1950.

97. Euler, U. S. v. Hormones of the sympathetic nervous system and the adrenal medulla. Brit. M. J., **20/1**: 105–108, 1951.

98. Euler, U. S. v. Unpublished results.

99. Euler, U. S. v. and Åström, A. Liberation of histamine and sympathin by stimulation of isolated splenic nerves from cattle. Acta physiol. Scandinav., **16**: 97–104, 1948.

100. Euler, U. S. v. and Hamberg, U. Colorimetric determination of noradrenaline and adrenaline. Acta physiol. Scandinav., **19**: 74–84, 1949.

101. Euler, U. S. v., Hamberg, U. and Hellner, S. Hydroxytyramine in normal human urine. (To be published).

102. Euler, U. S. v. and Hellner, S. Excretion of noradrenaline, adrenaline and hydroxytyramine in urine. Acta physiol. Scandinav., **22**: 161–167, 1951.

103. Euler, U. S. v. and Hellner, S. Increased urinary output of noradrenaline and adrenaline in muscular exercise. Acta physiol. Scandinav., 1951 (to be published).

104. Euler, U. S. v. and Hökfelt, B. Influence of hypophysectomy upon the distribution of noradrenaline and adrenaline in the spleen of the rat. Endocrinology, **48**: 98–100, 1951.

105. Euler, U. S. v. and Luft, R. The effect of adrenocorticotrophic hormone (ACTH) and adrenocorticotrophically active peptides (ACTH PEPTIDES) on the circulating eosinophils and urinary excretion of adrenaline and noradrenaline in a human subject. Acta Endocrinologica, **3**: 323–330, 1949.

106. Euler, U. S. v. and Luft, R. Noradrenaline output in urine after infusion in man. Brit. J. Pharmacol., **6**: 286–288, 1951.

107. Euler, U. S. v. and Schmiterlöw, C. G. Sympathomimetic activity in extracts of normal human and bovine blood. Acta physiol. Scandinav., **13**: 1–8, 1947.

108. Euler, U. S. v. and Sjöstrand, T. Bildung von Pressorsubstansen in der Niere. Naturwissenschaften, **31**: 145, 1943.

109. Farrerons-Co, F. J. Sobre el antagonismo regulador histamina-simpatina. Rev. esp. Fisiol., **4/1-2**: 57–71, 1948.

110. Fischer, P. and Lecomte, J. Nature des corps sympathicomimétiques dans les glandes parotides normales et énervées des crapauds tropicaux. Arch. internat. de Physiol., **57**: 277–285, 1950.

111. Folkow, B., Frost, J. and Uvnäs, B. Action of acetylcholine, adrenaline and nor-adrenaline on the coronary blood flow of the dog. Acta physiol. Scandinav., **17**: 201–205, 1949.

112. Folkow, B. and Uvnäs, B. The chemical transmission of vasoconstrictor impulses to the hind limbs and the splanchnic region of the cat. Acta physiol. Scandinav., **15**: 365–388, 1948.

113. Folkow, B. and Uvnäs, B. The chemical transmission of vasoconstrictor impulses to the hind limbs of the dog. Acta physiol. Scandinav., **17**: 191–194, 1949.

114. Frédéricq, H. Les nerfs cardio-régulateurs des invertébrés et la théorie des médiateurs chimiques. Biol. Rev. **22**: 297–314, 1947.

115. Gaddum, J. H. Gefässerweiternde Stoffe der Gewebe. Georg Thieme Leipzig, 1936.

116. Gaddum, J. H. Estimation of substances liberated by adrenergic nerves. Methods in Medical Research, Year Book Publishers, Chicago, **3**: 116–130, 1950.

117. Gaddum, J. H. and Goodwin, L. G. Experiments on liver sympathin. J. Physiol., **105**: 357–369, 1947.

118. Gaddum, J. H., Jang, C. S. and Kwiatkowski, H. The effect on the intestine of the substance liberated by adrenergic nerves in a rabbit's ear. J. Physiol., **96**: 104–108, 1939.

119. Gaddum, J. H., Khayyal, M. A. and Rydin, H. The release of pharmacologically active substances by nerve trunks during electrical stimulation. J. Physiol., **89**: 9–10, 1937.

120. Gaddum, J. H. and Kwiatkowski, H. Properties of the substance liberated by adrenergic nerves in the rabbit's ear. J. Physiol., **96**: 385–391, 1939.

121. Gaddum, J. H. and Lembeck, F. The assay of substances from the adrenal medulla. Brit. J. Pharmacol., **4**: 401–408, 1949.

122. Gaddum, J. H., Peart, W. S. and Vogt, M. The estimation of adrenaline and allied substances in blood. J. Physiol., **108**: 467–481, 1949.

123. Garb, S. Inotropic action of epinephrine, nor-epinephrine, and N-isopropyl-norepinephrine on heart muscle. Proc. Soc. Exper. Biol. & Med., **73**: 134–135, 1950.

124. Goldenberg, M., Apgar, V., Deterling, R. and Pines, K. L. Nor-epinephrine (arterenol, sympathin N) as a pressor drug. J. A. M. A., **140**: 776–778, 1949.

125. Goldenberg, M., Faber, M., Alston, E. J. and Chargaff, E. C. Evidence for the occurrence of nor-epinephrine in the adrenal medulla. Science, **109**: 534–535, 1949.

126. Goldenberg, M., Pines, K. L., Baldwin, E. F., Greene, D. G. and Roh, C. E. The hemodynamic response of man to norepinephrine and epinephrine and its relation to the problem of hypertension. Am. J. Med., **5**: 792–806, 1948.

127. Goodall, McCh. The presence of noradrenaline, adrenaline and an unknown sympathicolytic factor in cattle heart. Acta physiol. Scandinav., **20**: 137–151, 1950.

128. Goodall, McCh. Hydroxytyramine in mammalian heart. Nature, **166**: 738, 1950.

129. Goodall, McCh. Dihydroxyphenylalanine and hydroxytyramine in mammalian suprarenals. Åcta Chem. Scandinav., **4**: 550, 1950.

130. Goodall, McCh. Studies on adrenaline and noradrenaline in heart and suprarenals Acta physiol. Scandinav. Suppl. 85 to vol. **24**, 1951

131. Graham, J. D. P. Reflexly discharged sympathin in the cat. J. Physiol., **111**: 12P. 1950.

132. Graham, J. D. P. l-Adrenaline and l-noradrenaline. J. Physiol., **108**: 15P. 1949.

133. Graham, J. D. P. The actions of nor-adrenaline on the nonpregnant uterus of the cat in vivo. Arch. internat. de pharmacodyn et de thérap., **85**; 245–248, 1951.

134. Green, D. E. and Richter, D. Adrenaline and adrenochrome. Biochem. J., **31**: 596–616, 1937.

135. Greer, C. M., Pinkston, J. O., Baxter, J. H. and Brannon, E. S. Comparison of responses of smooth muscle to dl-β-(3/4-dihydroxyphenyl)-β-hydroxyethylamine (arterenol), l-epinephrine and "liver sympathin". J. Pharmacol. & Exper. Therap. **60**: 108–109, 1937.

136. Greer, C. M., Pinkston, J. O., Baxter, J. H. and Brannon, E. S. Nor-epinephrine (β-(3,4-dihydroxyphenyl)-β-hydroxyethylamine) as a possible mediator in the sympathetic division of the autonomic nervous system. J. Pharmacol. & Exper. Therap., **62**: 189–227, 1938.

137. Gross, F. Periphere Gefässwirkung von Adrenalin und Noradrenalin. Helv. Physiol. Acta, **7**: C43–C44, 1949.

138. Guggenheim, M. Die biogenen Amine. S. Karger, Basel and New York, 1940.

139. Guirin, S. and Delluva, A. M. The biological synthesis of radioactive adrenaline from phenylalanine. J. Biol. Chem., **170**: 545–550, 1947.

140. Hamberg, U. and Euler, U. S. v. Partition chromatography of adrenaline and noradrenaline. Acta Chem. Scandinav., **4**: 1185–1191, 1950.

141. Harley-Mason, J. The structure of adrenochrome and its reduction products. Experientia. **4**: 307–308, 1948.

142. Hartman, F. A. and Brownell, K. A. The adrenal gland. Lea & Febiger, Philadelphia, 1949.

143. Heirman, P. Modification des actions cardiaques de l'adrénaline au cours de son oxydation par les phénolases. Arch. internat. de Physiol., **46**: 404–416, 1938.

144. Henze, M. p-Oxyphenyläthylamin, das Speicheldrüsengift der Cephalopoden. Ztschr. f. physiol. Chem., Strassb., **87**: 51–58, 1913.

145. Heymans, C. and Heuvel-Heymans, G. van den. Action of drugs on arterial wall of carotid sinus and blood pressure. Arch. internat. de pharmacodyn. et de thérap., **83**: 520–528, 1950.

146. Heymans, C., Vleeschhouwer, G. R. de, and Heuvel-Heymans, G. van den. Adrenolytic drug and action of adrenaline and noradrenaline on carotid sinus. Arch. internat. de pharmacodyn. et de thérap., **85**: 188–193, 1951.

147. Hoffmann, F., Hoffmann, E. J., Middleton, S. and Talesnik, J. The stimulating effect of acetylcholine on the mammalian heart and the liberation of an epinephrine-like substance by the isolated heart. Am. J. Physiol., **144**: 189–198, 1945.

148. Holton, P. Noradrenaline in tumours of the adrenal medulla. J. Physiol., **108**: 525–529, 1949.

149. Holtz, P. Chemischer Nachweis der fermentativen Tyraminbildung durch Nierengewebe. Ztschr. f. physiol. Chem., **251**: 226–232, 1938.

150. Holtz, P. Ueber die sympathicomimetische Wirksamkeit von Gehirnextrakten. Acta physiol. Scandinav., **20**: 354–362, 1950.

151. Holtz, P., Credner, K. and Koepp, W. Die enzymatische Entstehung von Oxytyramin im Organismus und die physiologische Bedeutung der Dopadecarboxylase. Arch. f. exper. Path. u. Pharmakol., **200**: 356–388, 1942.

152. Holtz, P., Credner, K. and Kroneberg, G. Ueber das sympathicomimetische pressorische Prinzip des Harns ("Urosympathin"). Arch. f. exper. Path. u. Pharmacol., **204**: 228–243, 1947.

153. Holtz, P., Heise, R. and Lüdtke, K. Fermentativer Abbau von l-dioxyphenylalanin (Dopa) durch Niere. Arch. f. exper. Path. u. Pharmacol., **191**: 87–118, 1938–39.

154. Holtz, P., Kroneberg, G. and Schümann, H. J. Ueber das "Urosympathin" des Tierharns. Arch. f. exper. Path. u. Pharmacol., **209**: 364–374, 1950.

155. Holtz, P and Schümann, H. J. Karotissinusentlastung und Nebennieren. Arch. f. exper. Path. u. Pharmacol., **206**: 49–64, 1949.

156. Hoppe, J. O., Seppelin, D. K. and Lands, A. M. An investigation of the acute toxicity of the optical isomers of arterenol and epinephrine. J. Pharmacol. & Exper. Thérap., **95/4**: 502–505, 1949.

157. Horres, A. D., Eversole, W. J. and Rock, M. Adrenal medullary hormones in water diuresis. Proc. Soc. Exper. Biol. & Med., **75**: 58–61, 1950.

158. Humphreys, R. J. and Raab, W. Response of circulating eosinophils to nor-epinephrine, epinephrine and emotional stress in humans. Proc. Soc. Exper. Biol. & Med., **74**: 302–303, 1950.

159. Innes, I. R. and Kosterlitz, H. W. The effect of adrenaline and noradrenaline on the rate of the denervated heart. J. Physiol., **111**: 18P, 1950.

160. James, W. O. Demonstration and separation of noradrenaline, adrenaline and methyladrenaline. Nature, **161**: 851–852, 1948.

161. James, W. O. and Kilbey, N. Separation of noradrenalin and adrenalin. Nature, **166**: 67–68, 1950.

162. Kappert, A., Sutton, G. C., Reale, A., Skoglund, K. H. and Nylin, G. The clinical response of human beings to l-nor-adrenaline and its clinical applicability. Acta Cardiologica, **5**: 121–136, 1950.

163. Keller, E. B., Boissonnas, R. A. and Vigneaud, V. du. Origin of the methyl group of epinephrine. J. Biol. Chem., **183**: 627–632, 1950.

164. Kendall, E. C. Chemical studies of the suprarenal gland. J. Biol. Chem., **97**: Proc. IV, 1932.

165. Klopp, C. T. The reactions of partially denervated smooth muscle to adrenaline and sympathin. Am. J. Physiol., **130**: 475–480, 1940.

166. Konschegg, Th. Ursachen der Hypertonie. Wien. klin. Wchnschr., **269**–273, 1940.

167. Konzett, H. Sympathicomimetica und Sympathicolytica am isoliert durchströmten Ganglion cervicale superius der Katze. Helv. Physiol. Acta, **8**: 245–258, 1950.

168. Kroneberg, G. and Schümann, H. J. "Urosympathin". Arch. f. exper. Path. u. Pharmakol., **209**: 350–363, 1950.

169. Kwiatkowski, H. Histamine in nervous tissue. J. Physiol., **102**: 32–41, 1943.

170. Lands, A. M., Ludueña, F. P., Grant, J. I. and Ananenko, E. The pharmacologic action of some analogs of 1-(3,4-dihydroxyphenyl)-2-amino-1-butanol (ethylnorepinephrine). J. Pharmacol. & Exper. Therap., **99:** 45-56, 1950.

171. Lehmann, G. and Michaelis, H. F. Adrenalin und Arbeit. VII. Mitteilung. Die fluorimetrische Adrenalinbestimmung im Blutplasma. (Methodische Ergänzungen). Arbeitsphysiologie, **14:** 9-15, 1949.

172. Lerner, A. B. and Fitzpatrick, T. B. Biochemistry of melanin formation. Physiol. Rev., **30:** 91-126, 1950.

173. Lissák, K. Liberation of acetylcholine and adrenaline by stimulating isolated nerves. Am. J. Physiol., **127:** 263-271, 1939.

174. Lissák, K. Effects of extracts of adrenergic fibres on the frog heart. Am. J. Physiol., **125:** 778-785, 1939.

175. Lockett, M. F. The effect of denervation on the responses of the cat's nictitating membrane to sympathomimetic amines. Brit. J. Pharmacol., **5:** 485-496, 1950.

176. Loewi, O. Ueber humorale Uebertragbarkeit der Herznervenwirkung. Arch. f. d. ges. Physiol., **189:** 239-242, 1921.

177. Loewi, O. The Ferrier lecture on problems connected with the principle of humoral transmission of nervous impulses. Proc. Roy Soc. London, S. B. **118:** 299-316, 1935.

178. Loewi, O. Quantitative und qualitative Untersuchungen über den Sympathicusstoff. Pflügers Archiv., **237:** 504-514, 1936.

179. Loewi, O. Ueber den Adrenalingehalt des Säugerherzens. Arch. internat. de pharmacodyn. et de Thérap., **57:** 139-140, 1937.

180. Luco, J. V. and Goni, F. Synaptic fatigue and chemical mediators of postganglionic fibers. J. Neurophysiol., **11:** 497-500, 1948.

181. Luco, J. V. and Lissák, K. Chemical mediators in the aqueous humor. Am. J. Physiol., **124:** 271-278, 1938.

182. Lund, A. Simultaneous fluorimetric determinations of adrenaline and noradrenaline in blood. Acta pharmacol. et toxicol., **6:** 137-146, 1950.

183. Lundholm, L. The effect of l-noradrenaline on the oxygen consumption and lactic acid content of the blood in the rabbit. Acta physiol. Scandinav., **21:** 195-204, 1950.

184. McDowall, R. J. S. The nervous control of the blood vessels. Physiol. Rev., **15:** 98-174, 1935.

185. McDowall, R. J. S. The production of an adrenaline-like substance by the heart. J. Physiol., **104:** 41P, 1945.

186. Mann, M. Sympathin and the rat uterus. J. Physiol., **110:** 11P, 1949.

187. Mann, M. The nature of uterine sympathin in the rabbit. J. Physiol. In press.

188. Mann, M. and West, G. B. Uterine sympathin and noradrenaline. J. Physiol., **112:** 18P, 1951.

189. Mann, M. and West, G. B. The nature of hepatic and splenic sympathin. Brit. J. Pharmacol., **5:** 173-177, 1950.

190. Marple, C. D. The sympathetic control of the blood vessels of human skeletal muscle. Clin. Med.. Waukegan, **55:** 201-203, 1948.

191. Marquardt, P. Die Auf- und Abbaustufen des Adrenalins (in ihrer Bedeutung für Pharmakologie und physiologische Chemie). Enzymologia, **12:** 166-187, 1947.

192. Meier, R., Gross, F. and Eichenberger, E. Zur Differenzierung der Kreislaufwirkung sympathikomimetischer Substanzen. Helv. Physiol. Acta, **7:** 230-266, 1949.

193. Melville, K. I. The antisympathomimetic action of dioxane compounds (F883 and F933), with special reference to the vascular responses to dihydroxyphenyl ethanolamine (arterenol) and nerve stimulation. J. Pharmacol. & Exper. Therap. 317-327, 1937.

194. Middleton, S., Middleton, H. H. and Toha, J. Adrenergic mechanism of vagal cardiostimulation. Am. J. Physiol., **158:** 31-37, 1949.

195. Minz, B. La transmission chimique de l'influx nerveux. Flammarion, Paris, 1947.

196. Moeller, J. and Koppermann, E. Kreislaufreaktionen auf Arterenolinfusionen bei Normotonikern und bei Hypertonikern. Ztschr. Kreislaufforsch., **39:** 333-346, 1950.

197. Morton, M. C. and Tainter, M. L. Effects of sympathomimetic amines on perfused blood vessels. J. Physiol., **98:** 263-282, 1940.

198. Muralt, A. v. Die Signalübermittlung im Nerven. Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften 14. Reihe der experimentellen Biologie, Bd. III, 1946.

199. Nasmyth, P. A. The effect of some sympathomimetic amines on the ascorbic acid content of rats' adrenal glands. J. Physiol., **110:** 294-300, 1949.

200. Nickerson, M. and Goodman, L. S. Pharmacological properties of a new adrenergic blocking agent: N,N-dibenzyl-β-chlorethylamine (dibenamine). J. Pharmacol. & Exper. Therap., **89:** 167-185, 1947.

201. Norlander, O. Ether soluble compounds of adrenaline and noradrenaline with lecithin. Acta physiol. Scandinav., **21:** 325-331, 1950.

202. Page, I. H., Taylor, R. D. and Prince, R. Noradrenaline-like substance in blood demonstration by cross-circulation. Am. J. Physiol., **159:** 440-456, 1949.

203. Peart, W. S. The nature of splenic sympathin. J. Physiol., **108:** 491-501, 1949.

204. Raab, W. Epinephrine and related substances in human arterial walls and kidneys. Arch. Path., **35:** 836-845, 1943.

205. Raab, W. The pathogenic significance of adrenaline and related substances in the heart muscle. Exper. Med. & Surg., **1:** 188-225, 1943.

206. Raab, W. Adrenaline and related substances in blood and tissues. Biochem. J., **37:** 470-473, 1943.

207. Raab, W. Adreno-sympathogenic heart disease (neurohormonal factors in pathogenesis and treatment). Ann. Int. Med., **28:** 1010-1039, 1948.

208. Raab, W. and Humphreys, R. J. Secretory function of sympathetic neurones and sympathin formation in effector cells. Am. J. Physiol., **148:** 460-469, 1947.

209. Raab, W., Lepeschkin, E. and Gigee, W. Heart "Sympathin". Circulation, **1**: 741–752, 1950.
210. Richter, D. The inactivation of adrenaline in vivo in man. J. Physiol., **98**: 361–374, 1940.
211. Richter, D. and MacIntosh, F. C. Adrenaline ester. Am. J. Physiol., **135**: 1–5, 1941.
212. Rosenblueth, A. The transmission of sympathetic nerve impulses. Physiol. Rev., **17**: 514–537, 1937.
213. Rosenblueth, A. and Cannon, W. B. The chemical mediation of sympathetic vasodilator nerve impulses. Am. J. Physiol., **112**: 33–40, 1935.
214. Rosenblueth, A., Cannon, W. B., Rioch, D. McK., Cannon, B. and Sawyer, M. McK. A further study of vasodilators in sympathectomized animals. Am. J. Physiol., **108**: 599–607, 1934.
215. Ruiz-Gijón, J. Études sur l'oxydation de l'adrénaline. Arch. internat. de Physiol., **58**: 1950.
216. Ryd, H. SvenskKirurg. Fören. förhandl., 1951. To be published.
217. Scheiner, H. Action hypertensive des extraits de rate sur le chien atropinisé et cocainisé. Compt. rend. soc. de biol., **124**: 877–879, 1937.
218. Scheiner, H. Effects hypertenseurs produits par l'excitation du nerf splénique chez le chien atropinisé et cocainisé. Compt. rend. soc. de biol., **124**: 1219, 1937.
219. Schmiterlöw, C. G. The nature and occurrence of pressor and depressor substances in extracts from blood vessels. Acta physiol. Scandinav., **16**: suppl. 56, 1948.
220. Schmiterlöw, C. G. The formation in vivo of noradrenaline from 3:4-dihydroxyphenylserine (noradrenaline carboxylic acid). Brit. J. Pharmacol., **6**: 127–134, 1951.
221. Schümann, H. J. Ueber die Wirkung von Nebennierenextrakten auf Blutdruck und Blutzucker. Arch. f. exper. Path. u. Pharmakol., **206**: 475–483, 1949.
222. Selye, H. Stress. Acta, Inc. Med. Publ. Montreal, Canada, 1950.
223. Shaw, F. H. The estimation of adrenaline. Biochem. J., **32**: 19–25, 1938.
224. Shaw, F. H. and MacCallum, M. The possibility of the dual nature of sympathetic ganglion cells. Australian J. Exper. Biol. & M. Sc., **27/3**: 289–296, 1949.
225. Sivilla, S. V. and Monche, J. Simpatina E en sangre esplénica durante la hemorragia y la asfixia. Revista Esp. Fis. Barcelona, **4/1-2**: 103–108, 1948.
226. Smith, D. J. and Coxe, J. W. Comparative reactivity of isolated surviving coronary arteries to l-epinephrine and l-norepinephrine. Proc. Soc. Exper. Biol. & Med., **75**: 245–246, 1950.
227. Stehle, R. L. and Ellsworth, H. C. Dihydroxyphenyl ethanolamine (arterenol) as a possible sympathetic hormone. J. Pharmacol. & Exper. Therap., **59**: 114–121, 1937.
228. Stehle, R. L., Melville, K. I. and Oldham, F. K. Choline as a factor in the elaboration of adrenaline. J. Pharmacol. & Exper. Therap., **56**: 473–481, 1936.
229. Stein, Irwin D., Harpuder, K. and Byer, J. Effect of sympathectomy on blood flow in the human limb. Am. J. Physiol., **152**: 499–504, 1948.
230. Swan, H. J. C. Observations suggesting an inhibitory effect of adrenaline on sympathetic vasoconstrictor impulses in man. J. Physiol., **111**: 5P-6P, 1950.
231. Swan, H. J. C. Effect of noradrenaline on the human circulation. Lancet, **257**: 508–510, 1949.
232. Tainter, M. L. and Ludueña, F. P. Sympathetic hormonal transmission. Recent Progress in Hormone Research, **5**: 3–30, 1950.
233. Tainter, M. L., Tullar, B. F. and Ludueña, F. P. Levoarterenol. Science, **107**: 39–40, 1948.
234. Thibault, O. Adrénaline et sympathine. Action de la noradrénaline sur la consommation d'oxygéne du rat blanc. Compt. rend. Soc. de Biol., **142**: 47–50, 1948.
235. Tullar, B. F. The resolution of dl-arterenol. J. Am. Chem. Soc., **70**: 2067–2068, 1948.
236. Tullar, B. F. The separation of l-arterenol from natural U.S.P. epinephrine. Science, **109**: 536–537, 1949.
237. Uddén, P. Unpublished results.
238. Verly, W. Le réactif arsénomolybdique permet-il de reconnaitre l'adrénaline et l'artérénol dans les extraits tissulaires? Arch. internat. de Physiol., **55**: 397–408, 1948.
239. West, G. B. Quantitative studies of adrenaline and noradrenaline. J. Physiol., **106/4**: 418–425, 1947.
240. West, G. B. The estimation of adrenaline in normal rabbit's blood. J. Physiol., **106/4**: 426–430, 1947.
241. West, G. B. Further studies on sympathin. Brit. J. Pharmacol., **5**: 165–172, 1950.
242. West, G. B. Liberation of adrenaline from the suprarenal gland of the rabbit. Brit. J. Pharmacol., **5**: 542–548, 1950.
243. Williams, J. R. and Grossman, E. B. The recovery of an adrenalin-like substance from the kidney. Am. J. Physiol., **123**: 364–368, 1938.

THE INFLUENCE OF CERTAIN DRUGS ON BLOOD COAGULATION AND RELATED PHENOMENA*

WALTER H. SEEGERS

Department of Physiology and Pharmacology, Wayne University
College of Medicine, Detroit, Michigan

TABLE OF CONTENTS

I. Introduction 279
II. Methods for evaluating blood clotting phenomena and effects of pharmacologic agents 281
  Clotting of whole blood 281
  Prothrombin time 282
  Prothrombin 282
  Other quantitative methods 282
  Evaluating pharmacologic action 282
III. 3,3'-Methylenebis (4-hydroxycoumarin) or Dicumarol 283
  Chemistry 283
  Metabolism 284
  Liver 285
  New coagulation factors 286
  Clinical application 286
  Practical considerations 287
  Toxicity 288
  Thrombocytes 288
  Erythrocyte sedimentation rate 289
  Reproduction 289
  Miscellaneous 290
IV. Compounds related to Dicumarol 291
  3,3'-Ethylenebis (4-hydroxycoumarin) 292
  Compound 63 292
  Phenylindandione 293
  3 (α-acetonylbenzyl)-4 hydroxycoumarin 294
  3,3'-Carboxymethylene bis-(4-hydroxycoumarin) ethyl ester 294
V. Salicylates 296
VI. Methylxanthines 298
VII. Antibiotics 300
VIII. Protamines 301
IX. Toluidine blue 303
X. The cardiac glycosides 304
XI. Alpha tocopherol compounds 307
XII. Heparin and related substances 308
  Chemical nature 308
  Mechanism of action 309
  Administration 310
  Heparin substitutes 311

---

* Work incidental to the preparation of this review was aided by a grant from the National Institutes of Health, United States Public Health Service. I am indebted to Dr. John L. Fahey for his assistance with the section on drugs related to dicumarol and the material on salicylates, to Dr. Charles L. Schneider for the discussion of thromboplastin, and to Dr. Edna B. Andrews for assistance in arranging the material on vitamin K. Dr. Karl Paul Link of the University of Wisconsin and Dr. J. Garrott Allen of the University of Chicago reviewed the manuscript and I am very grateful for their help and interest.

THE INFLUENCE OF CERTAIN DRUGS ON BLOOD COAGULATION AND RELATED PHENOMENA*

WALTER H. SEEGERS

Department of Physiology and Pharmacology, Wayne University College of Medicine, Detroit, Michigan

TABLE OF CONTENTS

I. Introduction 279

II. Methods for evaluating blood clotting phenomena and effects of pharmacologic agents 281

- Clotting of whole blood 281
- Prothrombin time 282
- Prothrombin 282
- Other quantitative methods 282
- Evaluating pharmacologic action 282

III. 3,3′-Methylenebis (4-hydroxycoumarin) or Dicumarol 283

- Chemistry 283
- Metabolism 284
- Liver 285
- New coagulation factors 286
- Clinical application 286
- Practical considerations 287
- Toxicity 288
- Thrombocytes 288
- Erythrocyte sedimentation rate 289
- Reproduction 289
- Miscellaneous 290

IV. Compounds related to Dicumarol 291

- 3,3′-Ethylenebis (4-hydroxycoumarin) 292
- Compound 63 292
- Phenylindandione 293
- 3 (α-acetonylbenzyl)-4 hydroxycoumarin 294
- 3,3′-Carboxymethylene bis-(4-hydroxycoumarin) ethyl ester 294

V. Salicylates 296

VI. Methylxanthines 298

VII. Antibiotics 300

VIII. Protamines 301

IX. Toluidine blue 303

X. The cardiac glycosides 304

XI. Alpha tocopherol compounds 307

XII. Heparin and related substances 308

- Chemical nature 308
- Mechanism of action 309
- Administration 310
- Heparin substitutes 311

---

* Work incidental to the preparation of this review was aided by a grant from the National Institutes of Health, United States Public Health Service. I am indebted to Dr. John L. Fahey for his assistance with the section on drugs related to dicumarol and the material on salicylates, to Dr. Charles L. Schneider for the discussion of thromboplastin, and to Dr. Edna B. Andrews for assistance in arranging the material on vitamin K. Dr. Karl Paul Link of the University of Wisconsin and Dr. J. Garrott Allen of the University of Chicago reviewed the manuscript and I am very grateful for their help and interest.

XIII. Unrecognized nutritional factors 311
XIV. Vitamin K 312
Dicumarol-vitamin K antagonism 314
XV. Sulfonamides 315
XVI. Adrenocorticotropic hormone 315
XVII. Anesthetics and respiratory gases 316
XVIII. Epinephrine 318
XIX. Acetylcholine 319
XX. Blood coagulation components 320
Thrombin 320
Prothrombin 321
Streptokinase-Streptodornase 322
Thromboplastin 322
(a) General remarks 322
(b) Experimental pathology and relationship to pregnancy 322
(c) Effect on circulation 323
(d) Nature of fibrin deposits 325
Fibrinogen 325
XXI. Summary 326

References 327

I. INTRODUCTION

In recent years there has been a widening interest in the phenomena of blood coagulation and allied metabolic processes. This is partly due to the realization that cardiovascular diseases affect human welfare as much as or more than any other general class of ailments. A large proportion of cardiovascular difficulties involve the blood coagulation mechanism either from the standpoint of hemorrhages or intravascular clots. The enormous complexity of the chemical interactions in the phenomena of coagulation has required the attention of many people trained in various disciplines. There is now the general impression that real progress is being made and many are hopeful of gaining better control over many of the diseases that involve this mechanism. To this end drugs are becoming increasingly important and a review of their effects has long been needed. It must, however, be admitted that a thorough search of the literature offers only sporadic rewards so that it is necessary to describe or suggest one or more possible effects of certain drugs when one would like to be specific and conclusive.

Blood coagulation is only a part of the physiology of hemostasis and only one of the factors which contribute to the problem of thrombosis and related phenomena. This review does not include all of these considerations which embody a large amount of literature. The aim has been to restrict the discussion to the pharmacological effects of certain drugs on those factors which are directly concerned with the physiology of blood coagulation. Only in certain instances have effects on vascular tissues been considered. The drugs reviewed are: dicumarol, compounds related to dicumarol, salicylates, methylxanthines, antibiotics, protamines, toluidine blue, the digitalis series, alpha tocopherol compounds, heparin and related substances, unrecognized nutritional factors, vitamin K, sulfonamides, adrenocorticotropic hormone, anesthetics and respiratory gases, epinephrine and acetylcholine. In addition, a number of the substances which

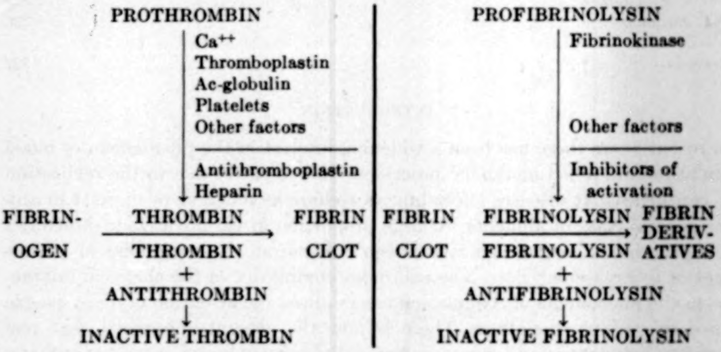
XIII. Unrecognized nutritional factors 311
XIV. Vitamin K 312
  Dicumarol-vitamin K antagonism 314
XV. Sulfonamides 315
XVI. Adrenocorticotropic hormone 315
XVII. Anesthetics and respiratory gases 316
XVIII. Epinephrine 318
XIX. Acetylcholine 319
XX. Blood coagulation components 320
  Thrombin 320
  Prothrombin 321
  Streptokinase-Streptodornase 322
  Thromboplastin 322
    (a) General remarks 322
    (b) Experimental pathology and relationship to pregnancy 322
    (c) Effect on circulation 323
    (d) Nature of fibrin deposits 325
  Fibrinogen 325
XXI. Summary 326

References 327

I. INTRODUCTION

In recent years there has been a widening interest in the phenomena of blood coagulation and allied metabolic processes. This is partly due to the realization that cardiovascular diseases affect human welfare as much as or more than any other general class of ailments. A large proportion of cardiovascular difficulties involve the blood coagulation mechanism either from the standpoint of hemorrhages or intravascular clots. The enormous complexity of the chemical interactions in the phenomena of coagulation has required the attention of many people trained in various disciplines. There is now the general impression that real progress is being made and many are hopeful of gaining better control over many of the diseases that involve this mechanism. To this end drugs are becoming increasingly important and a review of their effects has long been needed. It must, however, be admitted that a thorough search of the literature offers only sporadic rewards so that it is necessary to describe or suggest one or more possible effects of certain drugs when one would like to be specific and conclusive.

Blood coagulation is only a part of the physiology of hemostasis and only one of the factors which contribute to the problem of thrombosis and related phenomena. This review does not include all of these considerations which embody a large amount of literature. The aim has been to restrict the discussion to the pharmacological effects of certain drugs on those factors which are directly concerned with the physiology of blood coagulation. Only in certain instances have effects on vascular tissues been considered. The drugs reviewed are: dicumarol, compounds related to dicumarol, salicylates, methylxanthines, antibiotics, protamines, toluidine blue, the digitalis series, alpha tocopherol compounds, heparin and related substances, unrecognized nutritional factors, vitamin K, sulfonamides, adrenocorticotropic hormone, anesthetics and respiratory gases, epinephrine and acetylcholine. In addition, a number of the substances which are a part of the blood coagulation mechanism itself have become available commercially and are used for therapeutic purposes. They may thus be regarded as drugs and are briefly discussed from the standpoint of their pharmacological action. It is not possible to discuss this topic without presupposing familiarity with the blood coagulation mechanism itself. Since this field still presents a number of conflicting viewpoints, extensively reviewed (2, 18, 19, 39, 78, 85, 88, 101, 143, 147, 165–167, 169, 197, 206, 231, 257, 268, 280, 303, 320–322, 335, 356, 369, 377, 384, 387, 398, 412, 416, 432, 433, 487, 491, 503, 514, 515, 568, 571, 583, 589, 609, 610), it seems appropriate to present very briefly the concepts which the writer has used in evaluating the literature. For essentially the same reason a brief commentary seems necessary on several of the more common methods by which clotting may be investigated and by which pharmacological effects have been evaluated. A diagram representing some of the main chemical reactions in blood coagulation is given below.


PROTHROMBIN
Ca++
Thromboplastin
Ac-globulin
Platelets
Other factors
Antithromboplastin
Heparin
FIBRIN-OGEN
THROMBIN
THROMBIN
FIBRIN CLOT
+
ANTITHROMBIN
INACTIVE THROMBIN
PROFIBRINOLYSIN
Fibrinokinase
Other factors
Inhibitors of activation
FIBRIN CLOT
FIBRINOLYSIN
FIBRINOLYSIN
FIBRIN DERIVATIVES
+
ANTIFIBRINOLYSIN
INACTIVE FIBRINOLYSIN

The interpretation of the above diagram is as follows: The fibrinogen of the plasma is clotted by thrombin. The latter is derived only from prothrombin by the catalytic action of calcium ions, Ac-globulin, platelets, thromboplastin (thromboplastin, Howell; thrombokinase, Morawitz; cytozyme, Fuld and Spiro; tissue fibrinogen, Wooldridge; placental toxin, in the obstetric literature; thrombozyme, Nolf) and perhaps other factors. The Ac-globulin of the plasma is probably a proenzyme and is activated by small amounts of thrombin. The platelets furnish accelerator activity. They perhaps interact with plasma to furnish thromboplastin activity and they also contribute a factor which facilitates the interaction of thrombin and fibrinogen. Thromboplastin is primarily derived from the fixed tissues such as lung, muscle and brain. A large number of other factors have been described which are said to contribute to the activation of prothrombin, but it remains to be determined whether and how many such factors exist. The inhibitors of prothrombin activation probably counterbalance the activation mechanism. Antithromboplastin is considered to be present in the plasma and other tissues. Heparin which may be released from the cells probably acts as an inhibitor of prothrombin activation in conjunction with a plasma co-factor. In the same manner heparin probably exerts its antithrombic properties by acting in conjunction with a plasma co-factor. In addition, the plasma contains a substance, natural antithrombin, which can neutralize thrombic activity independent of heparin action. In the activation of prothrombin the following sequence of events may be suggested:

$$\text{Prothrombin} \xrightarrow{\substack{Ca^{++} \\ \text{Thromboplastin} \\ \text{Platelet Accelerator}}} \text{Thrombin} \tag{1}$$

$$\text{Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Serum Ac-globulin} \tag{2}$$

$$\text{Prothrombin} \xrightarrow[\text{Serum Ac-globulin}]{\substack{Ca^{++} \\ \text{Thromboplastin} \\ \text{Platelet Accelerator}}} \text{Thrombin} \tag{3}$$

In the first reaction the production of thrombin is considered to be slow; but as the small amount of thrombin activates plasma Ac-globulin the latter contributes to the acceleration of prothrombin activation. The third reaction represents, therefore, the rapid production of thrombin. The thromboplastin required for the initial production of thrombin, as represented by reaction one, is of fixed tissue origin. However, there is much evidence in the literature that the equivalent of thromboplastin may also be derived from the interaction of platelets and plasma (79, 252, 332, 339, 360, 410); this fact permits the above reactions to apply when blood is carefully drawn and not mixed with fixed tissue thromboplastin.

In addition to blood coagulation the diagram above depicts an enzyme mechanism for the dissolution of a clot. Fibrinolysin (plasmin) can dissolve a fibrin clot and produce derivatives of fibrinogen that do not respond to the action of thrombin. The fibrinolysin has its origin in plasma profibrinolysin (plasminogen). An activator of profibrinolysin is found in tissues and an inhibitor of the activation has only recently been described. Certain bacteria can also activate plasma fibrinolysin. The activity of fibrinolysin can be neutralized by antifibrinolysin which is a normal component of the plasma.

II. METHODS FOR EVALUATING BLOOD CLOTTING PHENOMENA AND EFFECTS OF PHARMACOLOGICAL AGENTS

Clotting of whole blood. The clotting time of the whole blood as generally estimated by the Lee-White method is a rough measure of the over-all clotting mechanism. It is influenced by variables introduced in drawing the blood and in doing the test. Because it is dependent upon the interaction of many factors and because of the unusually wide normal range, large abnormalities in one or more factors can be completely masked in the results of this test. For instance, the prothrombin concentration may be lowered to 20 per cent or below before prolonged clotting time is noticed. However, valuable data can undoubtedly be obtained when the method is employed meticulously by the same individual on repeated occasions.

Prothrombin time. The prothrombin time has most frequently been employed to report clotting data. There are many variations of the general procedure in which oxalated plasma is re-calcified and simultaneously a large amount of thromboplastic material is added. The clotting time is noted. To interpret the results it is assumed that one may judge the prothrombin concentration from the clotting time. This test is influenced by anticoagulation factors, but it has been fairly well established that the effect of these is minimized when the plasma is first diluted before the test is done. The procedure makes no allowance for the determination of concentrations of prothrombin exceeding 100 per cent of normal nor is it sensitive to changes in prothrombin concentration within the range of 50 to 100 per cent. Ac-globulin and perhaps other substances are controlling factors in the speed of prothrombin conversion to thrombin; consequently the test is susceptible to variations in Ac-globulin as well as to alterations in prothrombin reactivity. Changes in the so-called prothrombin time cannot with any certainty be ascribed to prothrombin alone, but most of the conclusions in the papers reviewed are based on the prothrombin time method.

Prothrombin. In a number of studies the two-stage procedure for the *quantitative* determination of prothrombin has been employed. There is considerable evidence that it gives specific information with regard to prothrombin concentration provided Ac-globulin is present in the plasma or supplied in the technical procedure.

Other quantitative methods. Quantitative measurements of heparin and fibrinogen concentration apparently give results which are quite reliable. Quantitative methods for Ac-globulin, platelet extracts, thromboplastin, antithromboplastin fibrinolysin, antifibrinolysin and thrombin have also been described in recent years but have as yet not been employed extensively. Most measurements of antithrombin activity recorded to date probably involved multiple factors.

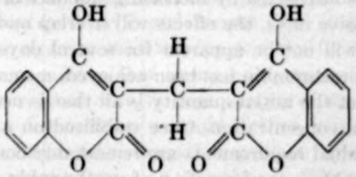
Evaluation of pharmacological action. The above comments are an expression of present day viewpoints concerning methods used in the study of the blood coagulation mechanism. Detailed critical evaluations have been presented elsewhere and further review at this time is not appropriate. However, because it is desirable to know what the specific effects of pharmacologic agents are, and because many of the reports in which nonspecific methods were used can only be evaluated now by saying that some sort of change occurred, or apparently did not occur, a plea must be made for thorough and competent investigations of the effects of therapeutic or toxic agents upon the individual components within the clotting mechanism *by means of methods in which the limitations are fully realized.* From the standpoint of evaluating pharmacologic action it is perhaps well to recall that alterations in the existing clotting mechanism of the living organism may take place in either the coagulant phase or the anticoagulant phase. The possibilities include alteration in the rate of physiological formulation or destruction of one or more of the significant substances participating in maintaining the fluid equilibrium. The same applies to anticoagulants. In the extreme, the effect may shuttle between complete removal of a coagulant and its potentiation, and between complete removal of an anticoagulant and its potentiation; of course, all combinations between these extremes are possible. Drugs may also act by entering into direct combination with a coagulant or anticoagulant or influence their release from storage depots. A brief outline of some principal mechanisms will serve to organize these viewpoints concerning the alteration of blood coagulation.

A. Coagulant effect
   1. Increased metabolic production of a coagulant.
   2. Decreased metabolic production of an anticoagulant.
   3. Direct interference with or destruction of an anticoagulant.
   4. Release of stored coagulant.
   5. Limited dilution of blood to make anticoagulants relatively less effective.

B. Anticoagulant effect
   1. Decreased metabolic production of one or more coagulants.
   2. Increased metabolic production of an anticoagulant.
   3. Direct inactivation or destruction of a coagulant.
   4. Release of stored anticoagulant.

III. 3,3′-METHYLENEBIS (4-HYDROXYCOUMARIN), DICUMAROL (BISHYDROXYCOUMARIN, U.S.P.)

Chemistry. The early history of dicumarol is associated with the observation of Schofield and Roderick (303) that the cause of a hemorrhagic and often fatal disease in cattle was the spoiled sweet clover hay on which they fed, and the demonstration by Roderick (303) that the cause of the hemorrhages was a severe reduction in the prothrombin in the circulating blood. In 1940 Link and his associates (303) not only isolated the active agent from spoiled sweet clover as a white crystalline material, but also synthesized it and described many of its metabolic effects (304, 305). This substance, 3,3′-methylenebis (4-hydroxycoumarin) has the following chemical structure:



Dicumarol is only slightly soluble in water, but forms various salts with strong alkalis that are soluble near pH 8. Its concentration in biological fluids can be measured by first extracting with heptane. After returning the drug to alkaline aqueous solution, the spectrophotometer can be used for quantitative measurements at 315 mμ (27, 503).

Metabolism. Dicumarol is effective after either oral or intravenous administration. The rate of absorption from the gastrointestinal tract is slow and varies from one individual to another and with the dosage. Six to 24 hours may be required to reach peak plasma levels. A threshold of five to ten mg. per liter of plasma must be reached before the prothrombin concentration falls, and this drop usually lags 24 to 48 hours behind the time of the peak plasma dicumarol concentration. In spite of variations in absorption there is no appreciable difference in the speed of action after administration by the intravenous as compared to the oral route. In either case, the drug is distributed to various tissues. It is found in liver, lung, and kidney, but not in appreciable amounts in the urine. Nevertheless it has been reported that the effect of the drug is prolonged and intensified by experimental renal damage (67, 449). The spleen, heart muscle and skeletal muscle contain only small amounts (503). That portion of the drug which is distributed to the plasma is almost completely bound to proteins and is removed only slowly, the time required being about 24 to 48 hours (503).

The response of different individuals to the drug varies, but seems to be the same in a given individual at different times. *Each individual is, therefore, observed when the drug is used, by analyzing the clotting status of the blood.* Individual variations in response seem not to be related to age, weight and sex, and perhaps only slightly to the diet. Interestingly, Link and his associates report that in rabbits susceptibility to dicumarol is an inherited Mendelian recessive characteristic. On continued or repeated dicumarol administration there is no development of tolerance or of increased susceptibility to the drug. Dicumarol has been given continuously for five years to rabbits (Link) and for more than three years to human beings (60) without any toxic effect.

The rapidity with which a single dose of dicumarol exerts maximal activity is a function of its quantity only when rather limited amounts of the drug are given; that is, as dosage is increased from minimal amounts, the rate of prothrombin fall increased slightly until a certain fixed slope of fall is reached, and further increase in the amount of dicumarol administered has no effect. However, the intensity of prothrombin reduction and the period required for return to normal prothrombin levels are definitely related to the amount of dicumarol administered, and are intensified by increasing amounts of the drug. If the dose is repeated on successive days, the effects will overlap and become cumulative, but the total effect will not be apparent for several days. Once a desired low therapeutic level of prothrombin has been achieved, a smaller dose of the order of 20 to 60 per cent of the initial quantity is all that is needed for maintenance of a low prothrombin concentration. Once stabilized on a maintenance dose of dicumarol, the individual requirements are remarkably constant. With common clinical therapy probably some formation of prothrombin continues and two to four days are required to reach equilibrium at about a 15 per cent level. As the effect of dicumarol subsides, the recovery of normal prothrombin levels is a somewhat slower process, requiring five to ten days (62).

The actual means by which dicumarol alters the mechanism of prothrombin formation in the liver is not known. The best estimates show that there are about 20 mg. of prothrombin per 100 cc. of plasma (485), and there are probably no other stores available save those in the lymph. Consequently, when prothrombin production is stopped the reserves are quite limited and become depleted rapidly by unknown mechanisms. Purified prothrombin, injected intravenously into animals depleted of prothrombin by means of dicumarol, largely disappears from the blood within 24 to 48 hours (343). Dicumarol has no known direct effect on prothrombin itself, except that it tends to stabilize purified preparations *in vitro* (495) under certain conditions. It has been suggested that an altered prothrombin is produced by dicumarol, but the evidence is not conclusive because the data are capable of being interpreted in another way (290). The idea, however, has possibilities in view of the demonstration that the activity of purified prothrombin can be altered in several ways (340, 488, 493).

Attempts to gain deeper insight into the details of dicumarol metabolism have only recently been made. Lupton (319) perfused excised rat livers and showed that the livers of dicumarol-treated rats do not add prothrombin to blood perfused through them. Jaques and associates synthesized dicumarol containing C^{14} in the methylene bridge, and gave it intravenously to mice and rabbits (287, 288, 529). None of the radioactivity appeared in the expired CO_2, and no significant amounts were in the lungs. It was found in large amounts in liver, intestine, gallbladder, feces and urine. The material in the liver was identified as dicumarol itself, which shows that there is a tendency toward fixation of the drug in that organ where it probably acts to interfere with prothrombin production. The time sequence between the prothrombinopenia of the blood and fixation of C^{14} in the liver indicates a direct relationship between the presence of C^{14} in the liver and the cessation of prothrombin production. Radioactivity appeared in the urine later after injection and was confined to metabolic products of dicumarol rather than to dicumarol itself. In the mouse some C^{14} was excreted in the feces. In man it has also been found that metabolic products appear in the urine (602).

Liver. There is no liver damage produced by therapeutic or even larger doses of dicumarol. This could by no means have been anticipated, although Link (63) points out that the conclusion was already implicit in the work of Schofield and Roderick. Consequently there are many papers on the subject, but since they have been reviewed by Marple and Wright (335) and by Link (306) there is no special purpose in again marshalling the evidence for the above conclusion. There is, however, a possibility that species differences in response exist for Jansen (242) was able to produce fatty degeneration of the liver by giving large doses to rabbits; the degeneration resembled that produced by chloroform intoxication. The conclusion also depends somewhat upon what is meant by liver damage for the concept cannot be all inclusive. Fibrinogen is produced by the liver and in dogs it is possible to cause either an increase or decrease in the plasma concentration of that protein by selecting the proper dose of dicumarol (239). Likewise, Ac-globulin which is most likely produced by the liver (546) tends to be depressed when dicumarol is first administered (157); but, despite continued administration, the plasma Ac-globulin concentration returns to normal, and its initial depression probably does not imply liver damage. It is quite possible to imagine metabolic readjustments that are reflected in temporary changes in changes in fibrinogen and Ac-globulin concentration. The cells in which the manufacture of prothrombin abruptly stops are not mechanical robots, but a kind of solution in which molecules are constructed, degraded and moved around, and perhaps the manufacture of fibrinogen and Ac-globulin is a certain kind of activity not entirely independent of prothrombin production. It is also interesting that mild trauma to the liver will cause plasma prothrombin concentration to fall (316). These viewpoints are not to be confused with the effect of liver damage, due to other causes, on the action of dicumarol (449).

New coagulation factors. The effects of dicumarol on the coagulation mechanism are apparently not restricted to prothrombin alone. The effect on antithrombin is a controversial question probably for the reasons mentioned above concerning the multiple factors which influence the antithrombin tests used to date. A group of factors are all described as being deficient in dicumarolized plasma or serum and are said to be related in some way to the activation of prothrombin. These include the following: SPCA (serum prothrombin conversion accelerator) (Alexander, 3), SPCF (serum prothrombin conversion factor) (Jacox, 240), prothrombin conversion factor (Owen and Bollman, 395), thrombotropin (Andreenko, 14), cothromboplastin (Mann and Hurn, 331), prothrombin accelerator (MacMillan, 330), proconvertin (Owren, 399), H-factor (Sorbye, 523), a new blood coagulation phase, (Copley, 119), and the factor (H-factor) in vitamin K deficient plasma which accelerates the coagulation of dicumarolized plasma (524). Possibly the aberrant behavior of Russell viper venom as thromboplastin can also be ascribed to a new factor (607). These have all been related to the conversion of prothrombin. Because further information on several of these factors is not available, comparison is hazardous, but it may be suggested that several and perhaps all of these factors are the result of different approaches to and investigations of the same phenomena. The data seem to indicate, however, that dicumarol produces an alteration in the clotting mechanism in addition to the prothrombin deficiency. Whether this alteration is a deficiency of a factor which is formed in the body separately from prothrombin or whether it is a factor derived from prothrombin in the process of thrombin formation has not been clearly established. From some of the data it is also by no means certain that the effect is not on the interaction of fibrinogen and thrombin.

Clinical application. Experience with the use of this drug has established the fact that there is definitely *a relationship between coagulability of the blood and thromboembolic phenomena and that much benefit is derived from the use of anticoagulants* (6, 32, 34, 66, 74, 75, 87, 183, 205, 212, 217–219, 282, 320, 329, 337, 338, 380, 392, 413, 414, 502, 609, 620–624). This important fact is also supported by the prior research on heparin (257, 373, 374, 521). Allen and Barker (4) have outlined the conditions in which artificially impaired coagulation of the blood is clinically of benefit, as follows: (1) thrombosis of coronary arteries, (2) thrombosis of cerebral arteries, (3) thrombosis of thrombophlebitis, (4) pulmonary embolism, (5) thrombosis of arteriosclerosis obliterans, (6) thrombosis of thrombo-angiitis obliterans, (7) peripheral arterial embolism, and (8) venous thrombosis of non-inflammatory origin, as in polycythemia vera. Jorpes (257) has also reviewed the social and economic aspects of anticoagulant therapy.

Practical considerations. It is not the purpose of this review to discuss the practical uses of the drug. It does, however, seem appropriate to mention again that *the variable response of individuals requires that adequate control measures be instituted*. All are agreed on this point. From extensive experience several viewpoints have developed concerning the kind of analyses that should be done on plasma. Some feel that the one-stage prothrombin time on the plasma is sufficient. The chief support for this argument is the view that, although prothrombin is not measured accurately by this technic, the test reveals the over-all coagulability status of the blood and that this is precisely the information a physician needs. Others have favored the view that the prothrombin time is more properly done with the use of diluted plasma because this gives a more accurate indication concerning the concentration of prothrombin and because the inhibitors of the blood are diluted sufficiently to eliminate their influence. Since dicumarol alters prothrombin concentration it is logical that quantitative measurement of prothrombin concentration (two-stage analysis) should give a more reliable means of controlling therapy. It has also been advocated that two methods be employed simultaneously. The general viewpoint has been that the two-stage method for the determination of prothrombin concentration is too complicated and time consuming. Experience by Olwin (60) has extended over a number of years and has involved the simultaneous use of both two-stage analysis and the prothrombin time test on undiluted plasma. In his experience use of the two-stage method is not out of the question from the standpoint of convenience or cost. He found wide variations between the methods used and believes the two-stage method to be more accurate and safer, but he also obtained valuable help from the results of the simpler method.

There seems to be no definite agreement as to the exact level to which prothrombin should be reduced for best therapeutic results. Some consider that the prothrombin level should be as low as possible, but short of producing hemorrhage. The dogmatic statement (430) has been made that the hemorrhagic tendency becomes very apparent when the concentration of prothrombin falls below 10 per cent, but a review of the clinical experience indicates that this viewpoint is altogether too rigid. It has even been said that there may not be a sharp correlation between the incidence of hemorrhage and the amount of dicumarol administered or between the prothrombin time and the incidence of bleeding (423). A case of accidental over-dosage with dicumarol was studied during the recovery phase (564). There was poor correlation between the bleeding tendency and the results of prothrombin time measurements. In experimental thrombosis, with dogs as subjects, there was a tendency for thrombosis when the prothrombin level (one-stage) was above 40 per cent (367).

The problems concerned with measuring the effects of dicumarol have been numerous (35, 46, 72, 234, 235). It was difficult to find a suitable thromboplastin. There were uncertainties as to whether the results should be reported in terms of per cent prothrombin "concentration" or in terms of clotting times. Often it was uncertain whether clotting times were being reported for diluted plasma or for undiluted plasma. Sometimes the results were adjusted in terms of control determinations and sometimes not. It has also been cautioned that a careful check of laboratory procedure might reveal errors in technic and terminology (415). It is almost surprising that all this confusion and use of empirical methods produced any definite concept at all.

Toxicity. The intravenous injection of lethal doses (dogs) resulted promptly in fatal circulatory collapse without evidence of hypoprothrombinemia (585). Splanchnic vasodilatation was observed in mice and hyperglycemia in rabbits. There was acceleration of the metabolic rate in rats and a rise of rectal temperature in both dogs and rats. Death from dicumarol uniformly occurs after a few weeks in rabbits given daily intravenous injections of 1 to 2 mg. per kg., in dogs given daily oral doses of 5 to 50 mg. per kg., and in mice and rats after ingestion of 0.01 to 1 per cent dicumarol in food (455). In other experiments in dogs with lesser amounts of dicumarol but still far larger than necessary or desirable for therapeutic purposes, the outstanding morphological changes were: (1) hemorrhages, gross or microscopic, (2) toxic lesions of small vessels, (3) acute renal glomerular swelling, and (4) toxic lymphoid tissue reaction. No necrosis of the liver or other consistent hepatic degenerative lesions were found (98). There was no effect on the extent of healing of experimental myocardial infarctions in dogs, produced by ligation of the anterior descending branch of the left coronary artery (291). In essentially similar experiments there was no effect on the electrocardiogram that could be ascribed to dicumarol (45), and no effect on the healing process or on the development of collateral circulation (65). It has been stated that the favorable effects of the anticoagulant upon coronary occlusion might be due to its action in increasing the coronary flow, but the data are not extensive (195). Capillary fragility tests show no changes when dicumarol is administered (263).

With the introduction of the drug it was inevitable that complications should be encountered from overdosage, self-medication, and other errors. The chief findings have been hemorrhage, severe headache, and bloody stools. Perhaps hematuria may be regarded as one of the first signs of impending danger from hemorrhage (91, 150, 151, 207, 302, 357, 627). A recent report based on a thorough study, but limited to one-stage prothrombin time tests, indicates that bleeding may occur when prothrombin deficiency is not great, that it may fail to occur when the prothrombin deficiency is marked, and that there is more apt to be bleeding when the deficiency has lasted for several days, the highest incidence being between the sixth and tenth day (222).

Thrombocytes. Dicumarol does not alter the thrombocyte count. It does, however, decrease the adhesiveness of platelets. The latter point was established in studies involving *in-vitro* technics (530, 619), in experimental animals (130) in which injury of the vein was produced by the technic (374) of inserting a linen thread and then crushing the intima, and by the technic (54) in which a glass seal was inserted between the carotid artery and the jugular vein.

Erythrocyte sedimentation rate. The most recent studies of this subject show that dicumarol does not alter the erythrocyte sedimentation rate (310, 605). Some earlier reports (5, 158, 414, 622) indicated that there was a definite influence which would need to be taken into account when sedimentation rate serves as a guide in diagnosis or prognosis. Five healthy male medical students were dicumarolized without influencing the sedimentation rate (403), and it appears that the evidence is decidedly in favor of the conclusion that there is no effect produced by dicumarol (280).

Reproduction. The involved situation presented during the reproductive cycle makes it somewhat difficult to obtain a clear view of the effect of dicumarol. The normal physiology of gestation itself presents some oddities which need to be kept in mind. Those who have studied the prothrombin concentration during pregnancy with the prothrombin time methods find, in general, a progressive increase in prothrombin which reaches a high peak in late pregnancy and subsides to the normal level in the early puerperium (134, 172, 175, 303, 386, 563). The same trend has been found with the two-stage assay procedure (255), but the increases in prothrombin were by no means as pronounced, thus raising the unanswered question whether the one-stage method is giving a true indication of prothrombin or whether the effect must be ascribed to some other plasma component. In the human infant the concentration of prothrombin is low at birth (80) and tends to be further depressed between the second and sixth day of life (396). With this as a background the questions which arise involve the possible effect of dicumarol on the fetus and on post-partum bleeding, and the possible transmission of dicumarol in the milk to an infant already having a low prothrombin concentration.

In experiments with dogs it was found that the prothrombin level (prothrombin time) in normal pups is lower than in adult dogs; furthermore, they are more susceptible to dicumarol than are adult dogs. The feeding of dicumarol to the mother increases the hypoprothrombinemia of the young at birth (436). In spite of the low prothrombin levels only a few of the young die of hemorrhage. In an extensive experimental study with pregnant rabbits the fetuses died *in utero* when the dose of dicumarol produced an "unsafe" level of prothrombin in the mother for as few as two days (284). When the prothrombin level of the mother was maintained at a "safe" level the newborn rabbits revealed an extreme decrease of their prothrombin with a definite tendency to hemorrhage. The mothers, on the other hand, did not experience excessive intra-partum or post-partum hemorrhage. It was found that dicumarol affects the prothrombin level of the infant to a much greater extent than that of the mother (284). Although the above data are based on animal experiments, clinicians (335, 545) advise that extreme caution is essential if and when it is necessary to use dicumarol in pregnant women. From the standpoint of puerperal thrombosis careful studies seem to lead to the conclusion that the dangers from postpartum hemorrhages are not great. In 14 patients dicumarol was given in generous therapeutic doses at the beginning of labor and the prothrombin level reached the therapeutic range in due course. The blood loss of these patients was not significantly different from that of a control group (36). In another investigation essentially the same conclusion was reached (137).

The problem presented by dicumarol excretion in the milk was encountered many years ago. Field (175) states that Roderick suspected this effect in cattle, because a calf which appeared to be normal at birth died of hemorrhages before it was old enough to eat the spoiled sweet clover hay which was being eaten by the cow. Field conducted his own experiments with rats and concluded that dicumarol fed to the mother caused a profound hypoprothrombinemia in the pups. In another study (172) it was discovered that rats are unusually resistant to the action of dicumarol during the lactation period. It was mentioned that such animals have unusually large livers and that this might be related in some way to the resistance which these animals show. In one study with dogs there was definite indication that the milk contains a prothrombinopenic agent (436), but in another study the contrary conclusion was reached (463). Brambel and Hunter (73) and Brambel, Hunter and Fitzpatrick (76) discussed the implications for the human species, and carefully standardized prothrombin-clotting-time methods to study the prothrombin activity of the nursing infant. One-hundred and twenty-five nursing mothers received the drug in therapeutic doses and this had no effect on the infants as measured by the technics employed (73). Adamson *et al.* (1a) describe their successful use of dicumarol in pregnant patients and discuss the many factors which must be taken into account.

Miscellaneous. Dicumarol tends to prevent thrombus formation when blood is caused to sludge by experimental procedures, probably by prevention of the sludged masses of cells from becoming adherent to the endothelial lining of the blood vessel (286). Under certain experimental conditions it was found that rutin has no protective action in rats receiving dicumarol. The "defect" in the vessel walls which leads to hemorrhage as a result of dicumarol may not be the one with which rutin is concerned (108). With dicumarol the minimal requirement of calcium for optimal prothrombin time is increased (247). Animals receiving dicumarol during fever showed a tremendous increase in the prothrombin time (448). Rabbits, after dicumarol treatment, have been found to be more sensitive to intracutaneous injections of hemolytic streptococci than the control animals. It is suggested that the lack of fibrin in tissues is a factor in the spread of the infection, in contrast to the localization of the infection in the control animals (565). Clot retraction is not altered.

In some studies by Bose (69) it was concluded that the thromboplastic activity of rabbit brain is reduced by the administration of dicumarol. This conclusion was also reached in three other laboratories (370, 460, 534). Munro and Lupton (370), however, were not able to show such a reduction in activity in dog or rat brain tissue. The reviewer finds it difficult to believe that dicumarol can reduce the thromboplastic activity of rabbit brain and suggests that the data may eventually be reinterpreted.

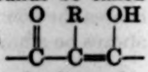
Guinea pigs on a scurvy-producing diet respond to dicumarol with a more extensive hypoprothrombinemia and it is of longer duration than in animals on a normal diet. The mere increase or decrease of vitamin C alone has no effect on prothrombin. It is thus considered that vitamin C has protective action against hemorrhage (42, 540). In human beings vitamin C deficiency also enhances the dicumarol effect (115) and it has been recommended that vitamin C always be administered with dicumarol (335).

IV. COMPOUNDS RELATED TO DICUMAROL

Since dicumarol has the advantage of being equally active orally and intravenously and entails little danger of toxic side effects at therapeutic levels, search began for substances which possessed these qualities without the disadvantages of the delay in becoming effective, of the prolonged recovery period, of the need for frequently repeated testing of prothrombin levels, and of the danger of bleeding from excessive dosage. The quest for other effective compounds served the additional purpose of delimiting the portions of the 3,3′-methylenebis (4-hydroxycoumarin) molecule essential for its action on the prothrombin formation mechanism.

Link and his associates carried out an extensive program of preparing derivatives (233, 236, 531, 532, 541), degradation products (232) and analogues of dicumarol and 4-hydroxycoumarin. These were tested and evaluated on the basis of response evoked by a *single oral dose* to standardized rabbits, with prothrombin time determinations on 12.5 per cent plasma at 24, 48, and 72 hours (394). It is evident that the effect of a rapidly acting compound or one which required repeated dosage for full effectiveness could be missed under the conditions employed for the *screening* tests.

From this study it was concluded that minimal requirements for pharmacological activity are an intact 4-hydroxycoumarin residue with a hydrogen atom or carbon residue substituted on the 3 position. For maximal activity a bis arrangement is required with a 1–5 spatial relationship between the enolic hydroxyl group of 4-hydroxycoumarin and a keto group. No substance was found to be as effective as dicumarol under the conditions of the assay with selected susceptible rabbits as test animals. Furthermore, diminished prothrombin activity was found only in those analogues of 3,3′-methylenebis (4-hydroxycoumarin) and related compounds which theoretically or actually yield salicylic acid or an *o*-hydroxybenzoic acid derivative on degradation (308). For this reason and because salicylates were shown to produce hypoprothrombinemia in experimental animals (308) and man (355, 499, 501), it was postulated that dicumarol may be metabolized to salicylic acid in the body and thus induces the hypoprothrombinemic effect (303). Evidence against this hypothesis consists in the effectiveness of salicylates being only about five to ten per cent that of dicumarol (308), in the failure to find salicylates in the urine after administration of dicumarol (292), and in the fact that the hypoprothrombin effect in liver is most likely due to unchanged dicumarol (287, 288).

Similar investigation of coumarin derivatives and analogues was undertaken by Meunier and Mentzer (348–352, 354, 362) who also were unable to find a compound with activity comparable to dicumarol. Emphasizing the structural similarity of many of these compounds to vitamin K and demonstrating that concomitant administration of the vitamin impaired their effectiveness, the above investigators proposed that dicumarol and allied compounds be called antivitamins K. Mentzer (348) suggested that the configuration  $-\overset{\overset{O}{\|}}{C}-\overset{\overset{R}{|}}{C}=\overset{\overset{OH}{|}}{C}-$ present in the active naphthoquinone, hydroxycoumarin and indandione derivatives is necessary for antivitamin K activity. Smith has emphasized that the nature of the substituent R must be defined in greater detail (512). However, Mushett and Seeler (375, 497) have demonstrated antiprothrombinemic activity on administration of 2-sulfanilamide quinoxaline, which does not bear the structure indicated above. It is, moreover, antagonized by vitamin K_1. This finding is interesting in the light of Roblin's (453) statement that not all important antagonists are structurally related to the affected metabolite. Although eventually sufficient evidence may be acquired that will show these compounds to be specifically antivitamin K, the present evidence only indicates that the effect of dicumarol and related compounds is in a general way opposed to the eventual metabolic effect of vitamin K.

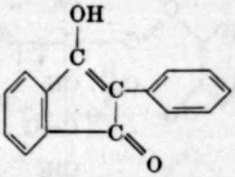
Smith (511–513) has investigated various phthiocol derivatives and found that, with high dosage *in vivo*, 3-hydroxy-naphthoquinones substituted in the 2 position with a hydrocarbon chain of six or more carbon atoms induced hypoprothrombinemia in rats. Vitamin K_1 was much more effective than menadione in preventing the onset of hypoprothrombinemia. No hepatic damage was noted with these compounds.

3,3′-Ethylenebis (4-hydroxycoumarin). Jansen and Jensen (243), Lehman (289), Fantl (160, 161), and Bayerle and Marx (43) also investigated substances structurally related to 3,3′-methylenebis (4-hydroxycoumarin) but found none so effective; the results, when identical compounds were investigated, are in substantial agreement with those reported by Overman *et al.* (394). Since 3,3′-ethylenebis (4-hydroxycoumarin) was found to rank closest to dicumarol in activity (about 25 per cent as active), clinical trial was made by Fantl and Nance (161) who describe a slightly more rapid action and a shorter recovery period than with dicumarol. The Australian workers (161) encountered great variations in response and a high percentage of resistance to the drug which could not be correlated with age, sex, pathological state or other treatment of the patients. Fibrinogen production was also decreased but it was not possible to ascribe this specifically to the drug because other factors may have been the cause.

Compound 63. Another substance with anticoagulant activity related to dicumarol is 2-methyl-2-methoxy-4-phenyl-5-oxodihydro-pyrano-(3,2-c) (1) benzopyran (designated No. 63) which, in rat, dog and man, appears to be two to three times more potent than dicumarol on a mg. per kg. basis (40). This 4-hydroxycoumarin was originally studied by L. D. Scheel in Link's laboratory (463a). For historical details the paper of Battle *et al.* (40) may be consulted. Optimal effects are achieved in 24–48 hours but a greater anticoagulant action and a longer duration without hemorrhagic manifestations are claimed. No toxic lesions in the small vessels were noted, as have been reported with unduly high dicumarol doses. Vitamin K counteracts the anticoagulant action of No. 63.

Phenylindandione. Kabat, Stohlmann and Smith (266), in a study of toxic effects of indandione compounds, noted incoagulability of the blood. Because their investigations were carried out with a one-stage technic of prothrombin analysis the distinction between prothrombin and Ac-globulin changes cannot be made. Both may have been altered. Only those compounds which contained three, instead of two, ketone groups were active. These were 2-pivalyl, 2-isovaleryl, 2-propionyl, and 2-naphthoyl compounds of 1,3-indandione. 2-Pivalyl was considered to be as effective as Dicumarol. These compounds were not effectively counteracted by vitamin K (2-methyl-1,4-naphthoquinone), but in some instances and under restricted circumstances some effect was noted. Toxic effects were principally hemorrhagic with large doses, although hepatic centrolobular fatty degeneration and focal necrosis were noted. In addition to the *in-vivo* effect of these compounds, 2-isovaleryl-1,3-indandione added *in vitro* to plasma accelerated the deterioration of prothrombin activity.

The structure of phenylindandione is as follows:



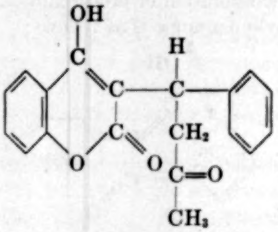
Phenylindandione was noted (353) to have a transitory effect on the prothrombin time. Soulier and Gueguen (527) further investigated this substance and later Jaques (253) and Blaustein (58, 59) and their associates also investigated its action. Phenylindandione has no effect *in vitro* and acts to lower prothrombin presumably by interfering with its formation in the liver. Ac-globulin appears not to be significantly altered, although an earlier report, corrected by the authors themselves (254), indicated a reduction in this component and several experiments of Kabat, Stohlmann and Smith (266), in which fresh serum restored coagulability to treated plasma, could be attributed to such an effect.

The rapid fall and recovery of plasma prothrombin contrast phenylindandione with dicumarol. Immediately after its administration, Jaques *et al.* (253) noted an unexplained prolonged prothrombin time lasting two to three hours. The major prolongation after a single dose begins at 10 hours, reaches a peak at about the 24th hour and returns to normal levels by the 40th hour. A single dose is much less effective than repeated administration at eight-hour intervals. On this schedule the drug is as effective as dicumarol in maintaining a prolonged prothrombin time. Recovery time after repeated dosage is of the order of 48–72 hours.

Phenylindandione is equally effective by oral and intravenous routes; it is excreted by the kidney (253). Vitamin K (2-methyl-1,4-naphthoquinone) in moderate doses does not alter the response to this drug. Individual variation in response has been noted, and resistance to it was seen in three of Blaustein's 53 patients (59). In many cases marked swings in prothrombin time values were noted.

Overdosage results in a hemorrhagic tendency, correctible within 24 hours by withdrawal of the drug. Toxic renal damage (mild fatty degeneration particularly in the loops of Henle) was noted after very large doses. Polydypsia, polyuria, dryness of the mouth and tachycardia have been noted (527) but these effects have not been confirmed (59). Therapeutic levels of the drug produce no change in erythrocytes, leukocytes, platelets, blood sugar, cholesterol, total protein, serum albumin or globulin, fibrinogen, sedimentation rate, hippuric acid formation, icteric index, van den Bergh reaction or bromsulphalein excretion.

3(α-acetonylbenzyl)-4-Hydroxycoumarin. This compound has the following structure:

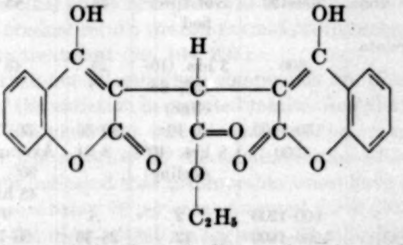


It was originally called compound 42 and is now known as warfarin (454a). This compound was one of about 300 appraised by Link's group, with the standardized rabbit assay (394). This study was later continued and the chemical was found to be of special interest because of species specificity in susceptibility to its action. It was found that the rat is especially affected and this suggested to Link and his associates (63) that the chemical might be useful in the control of rodents. In extensive field tests it has been found that the compound shows much promise as a rodenticide. The habits of this animal make it difficult to devise a particular bait which will not cause them to shy away. This apparently is not a difficulty with warfarin. Furthermore, the relatively low toxicity for other animals makes it uses in rat extermination relatively safe for those species which man desires to preserve.

3,3'-Carboxymethylene bis-(4-hydroxycoumarin) ethyl ester. In the report of Solomon (522) and associates it is stated that this compound was synthesized by Rosicky. It has been studied extensively in the laboratory and in the clinic (55, 84, 86, 139, 140, 142, 144, 192, 193, 268, 269, 271, 277, 297, 381, 426, 447, 480). The work of von Kaulla and his associates was especially thorough and extensive. The early clinical work was by Reinis and Kubik (447), Della Santa (129) and de Nicola (381).

Like dicumarol, this drug, called tromexan, acts by impairing the capacity of the liver to produce prothrombin. It has been stated that Ac-globulin is also lowered in concentration at the beginning of administration (480), but the main effect is apparently on prothrombin (142). As compared with dicumarol the response is more rapid and the return of the prothrombin time to normal is more rapid. Some observations in human beings are tabulated in Table 1.

Pulver and von Kaulla (426) found that tromexan unites with diazotized *p*-nitraniline at pH 6.0 to form a yellow dye and this is the basis of a quantitative color reaction. In the experimental animal and in man tromexan appears in the blood about as rapidly as dicumarol after oral ingestion, but it disappears more rapidly (36 hours vs. five days) (193). When its repeated administration is stopped, the normal prothrombin time is again reached in 48 hours, and the drug is no longer in the blood in detectible quantities after 36 hours. A certain minimum blood concentration is necessary to prolong the prothrombin time. Pulver and von Kaulla (426) found that, in the rabbit at least, the drug is partially eliminated unchanged in the urine, to a large extent as inactive tromexan acid A and to some extent as tromexan acid B. The structure of tromexan is as follows:



In the absence of pre-existing hepatic disease there is no effect on liver function (83, 84, 139, 447). However, pre-existing liver damage will alter the response to the drug and renal insufficiency prolongs its action (297) but the drug causes no impairment of renal function (83, 139, 447). There is no effect on red or white blood cell count (83, 84), sedimentation rate (83, 447) or platelet count (139). A transient fall in blood fibrinogen has been recorded (139).

Tromexan has a very favorable potency/toxicity ratio. Von Kaulla and Pulver made extensive toxicity experiments (271). The oral LD$_{50}$ after a single administration is approximately 750 mg. per kg. for mice and 1000 mg. per kg. for rabbits. After daily feeding of 100 mg. per kg. to mice death usually occurs within 26 to 51 days. At autopsy the above investigators found fatty infiltration of the liver and, less frequently, of the kidneys. In rats prolonged administration of large quantities also caused fatty infiltration of the liver. When the above toxic quantities are compared with the therapeutic doses in Table 1 it can be seen that there is a wide margin of safety.

V. SALICYLATES

The chemical similarity between salicylate and dicumarol and the discovery that salcylic acid was the primary degradation product of 3,3′-methylenebis (4-hydroxycoumarin) suggested to Link (303) that dicumarol might owe its effectiveness *in vitro* to conversion to salicylic acid. Investigation of this hypothesis (308) showed that salicylates under certain conditions produce hypoprothrombinemia. These conditions apparently were species susceptibility, limitation in vitamin K availability and administration of sufficiently large amounts of salicylate.

TABLE 1

Effect of a single dose of tromexan on prothrombin time in normal individuals

AUTHORS AND REFERENCES	TROMEXAN DOSAGE	PROTHROMBIN-TIME			METHOD OF DETERMINATION
		Detectable fall	Maximum fall	Return to normal	
	mg.	*hrs.*	*hrs.*	*hrs.*	
Reinis and Kubnik (447)	900	1–2	12–24	48	Legler's modification of Quick's method
Della Santa (139)	Not specified	3	20–24	48	Quick's method modified by Jeanneret
Della Santa and von Kaulla (140)	300–450	Not specified	24	48	Not specified
Bickel and Della Santa (55)	600	3 hrs. (10–15% decline)	24	48	Not specified
Deutsch (142)	150–1800	6–10	30–36	36–72	Not specified
Burt *et al* (86)	1200	8 hrs. (40% decline)	8–24	Average 90% at 48 hrs.	Not specified
Lian *et al.* (297)	600–1200	2	4	9	Not specified
Burke and Wright (83)	1200–1800	12	28–36	60–72	Link-Shapiro modification of Quick's method
Solomon *et al.* (522)	1200	8	10	24	Quick's method

Rats, human beings and rabbits appear to be more susceptible than dogs to the hypoprothrombinemic effect of salicylate (107, 308, 442). The suggestion that this low activity in dogs is due to rapid excretion (208) was investigated by producing anuria with uranium acetate in two dogs (176). In these animals salicylates alone were still unable to produce a hypoprothrombinemia. In dogs, salicylate effects were detectable only if production of prothrombin was concomitantly reduced by dicumarol administration. In rats on a low vitamin K diet (308), however, the salicylates do reduce the prothrombin level. This vitamin K restriction was regarded as essential to the effectiveness of the salicylates. The dog, in contrast to the rat, is an animal in which vitamin K deficiency is produced only with considerable difficulty. Thus it seems possible that the variation in species response to salicylates may be related to the state of vitamin K metabolism and storage. In rabbits (44, 307, 442) and human subjects (33, 89, 96, 138, 164, 215, 355, 397, 442, 499, 505), hypoprothrombinemia has been found without restriction of vitamin K. However, the reductions reported have generally been less than to 50 per cent of normal.

It is interesting to note that Jaques and Lepp (248) found that intravenous administration of salicylates to rabbits caused no alteration in prothrombin time, while oral administration resulted in a prolongation. After the oral administration of sulfasuxidine, the oral administration of sodium salicylate did not affect the prothrombin time. They suggest that salicylate may be converted to dicumarol or to a substance with similar prothrombinopenic properties by bacterial action in the intestinal tract. Exactly opposing results were reported by Meunier *et al.* (352) who found intravenous but not oral administration to be effective. In the rat only a time difference was noted in comparing the effectiveness of the two routes (308).

Large amounts are necessary to reduce the prothrombin, but these doses may be within the therapeutic range (111). Repeated doses are more effective than a single dose, the maximal effect being reached in about 48 hours (164, 355, 442) and followed by a gradual return toward normal prothrombin levels even under continued salicylate treatment (89, 164, 397).

The possibility that not all salicylate compounds are of equal activity may account for some of the variation in reported results. Acetylsalicylic acid, methyl salicylate, sodium salicylate and salicylic acid are the compounds most often used. Barclay (33) has reported on the administration of sodium paraaminosalicylate (PAS) and indicated that prothrombin times have a tendency to prolongation but none was below 70 per cent of normal. Link (307) observed acetylsalicylic acid to be the most potent among some 60 salicylates tested. Salicylic acid proved to have about five per cent of the activity of dicumarol. The testing methods, however, are subject to the same qualifications discussed previously regarding the evaluation of compounds chemically allied to dicumarol. Clark and Spitalny (107) found that equimolar doses of sodium salicylate, methyl salicylate and acetylsalicylic acid have about the same activity.

The metabolic state of the recipient may well have considerable effect upon the response to the salicylates. The role of vitamin K has been mentioned earlier. The prothrombinopenic action of sodium salicylate was greatly augmented by experimental hyperthermia induced by yeast injection or high environmental temperature, by hyperthyroidism and by daily alcohol administration (107). From these experiments on rats it was suggested that increased metabolic activity enhances the effect of salicylates.

The relationship to vitamin K was further probed. Link and coworkers (308) demonstrated that co-administration of synthetic vitamin K and salicylates would prevent the prothrombinopenic effects of the latter. Others observed the same effect (172, 355, 501, 505). Approximately one mg. of menadione will counteract the prothrombin-reducing action of one gm. of acetylsalicylic acid (505). The correction of hypoprothrombinemia can be achieved without altering the plasma level of salicylic acid (629).

In discussing the mechanism of salicylate action, Field, Spero, Link and others (176, 308) have emphasized that salicylates act only when the vitamin K function is impaired and that these agents have a low affinity on a competitive inhibition basis, compared to dicumarol, for the site of vitamin K action. They believe that the effect of salicylic acid on prothrombin synthesis is at a site somewhat different from that of dicumarol. They also suggest that there may be a direct intravascular effect which produces inhibition of prothrombin.

Previously Link *et al.* (308) had indicated that there was no *in-vitro* effect of salicylates. Stefanini and Petrillo (535) showed a retardation of prothrombin time when the salicylate concentration was greater than 200 mg. per cent. A deleterious effect on purified prothrombin was noted at high salicylate concentrations by Seegers, Loomis and Vandenbelt (495). The possibility of a direct effect is not excluded.

All investigations of *in-vivo* salicylate effects were carried out by one-stage technics for the determination of "prothrombin time". The use of whole plasma testing as described by Quick (435) was found to be relatively insensitive to the degree of change produced by these compounds (303) so that 12.5 per cent plasma was most extensively employed. Because the one-stage tests utilized here are susceptible to alteration in Ac-globulin as well as prothrombin, the change produced by the salicylates cannot with certainty be ascribed to reduction in either substance or in both. The prolongation of "prothrombin time" cannot be interpolated to mean a hypoprothrombinemia. Reports of salicylate effects based on more specific methods have not yet appeared.

Salicylates in sufficient dosage and under proper metabolic conditions do interfere with the coagulation mechanism (15, 109, 116, 117, 194, 199, 308, 379, 456, 537, 575). Other pharmacologic aspects of the salicylates have been reviewed by Smith (517).

VI. METHYLXANTHINES

Interest in these compounds was stimulated by the work of Link and his associates (303). When they were isolating dicumarol it was noticed that the concentrates were far more potent when freed of purine bodies. This increase in potency was out of proportion to what was expected on the basis of simple removal of presumably inert materials. They state that the literature as early as "1896 expressed the thought that caffein evoked the development of a coagulative ferment." Further pursuit of this problem supported the conclusion (174) that the methylxanthines, caffeine, theobromine and theophylline, given orally, induce a hyperprothrombinemia in the dog, rat and rabbit. These drugs could lessen the hypoprothrombinemic effect of dicumarol, and this action was not shared by other purines and was not thought to be vitamin K-like. They suggested that the methylxanthines might exert functional stimulation of hepatic tissue, but in a later communication Link (60) fully clarified his position by stating that this is only a suggested explanation and that the term "hyperprothrombinemia" is quite expendable. In the main their work has withstood the test of time, but there are some aspects which require further consideration.

In man, as early as 1920, Nonnenbruck and Szyszka (385) found that methylxanthines caused a definite shortening of the clotting time of the whole blood. It was suggested that they might be used to aid the hemostatic mechanism when desirable. This report caused Addicks (1) to do studies on man and the original work was confirmed. In rabbits a reduction in whole blood clotting time was also found (344). Apparently the bleeding time in rabbits is also reduced (510). In man Scherf and Schlachman (465) found the whole plasma coagulation time to be shortened. However, Blood and Patterson (64) found no change in the whole blood coagulation time in man. This disagreement makes it difficult for a reviewer, for the overwhelming evidence indicates that the whole blood clotting time *is reduced* by these drugs. Blood and Patterson do not quote the prior literature and did their tests at the end of one hour, whereas the main effect apparently comes in about three hours. Their test subjects were also not in good health. Nevertheless they have the support of another study (196) in which the capillary tube method was used for studying whole blood clotting time.

In 1945 Quick conducted experiments in dogs (434) and found no evidence that caffeine, theobromine and theophylline, fed in large doses, increase the prothrombin level of the blood. The drugs also did not antagonize the action of dicumarol. He suggested that his results differed from those of Link and associates because the latter determined prothrombin times with the use of 12.5 per cent plasma. This explanation, as will be seen later, is not adequate. In human patients receiving large therapeutic doses, the various prothrombin time methods give different results (64, 77, 196, 224, 391, 451, 465) but for the most part there are no changes observed. Positive evidence was obtained when Russell viper venom was used as thromboplastin (465). The significance of this is not clear, and it would seem to require further study. From a study conducted with a critical attitude toward methods, it was also concluded that hyperprothrombinemia is only apparent (383). If one assumes that these methods give essentially negative results with human plasma, it is necessary, in view of the results on whole blood clotting time and other experiments to be discussed below, to conclude that they are not adequate for disclosing what the methylxanthines do to the clotting mechanism. Olwin (60), who has had extensive experience with blood coagulation methods and dicumarol therapy, states categorically that human beings require much more dicumarol when they are also receiving methylxanthines.

In animal experiments Honorato (227) was able to show that the administration of caffeine, theobromine and theophylline and sodium benzoate causes an increase of his plasmatic co-factor of thromboplastin (Ac-globulin) (226). During the same year and independently, McCormick and Young (342) showed that Ac-globulin concentration shows a marked and persistent rise when aminophylline is given to dogs in large doses. They used the quantitative method for Ac-globulin analysis devised by Ware and Seegers (596). There was no shortening of the whole blood coagulation time which is no surprise because it is normally very short in the dog. They found the prothrombin concentration as measured by two-stage methods (592, 596) to be elevated slightly, after which it was lowered somewhat below normal before returning to normal. By these more specific methods they were thus able to uncover a complex situation in which an elevated Ac-globulin concentration was the predominant feature. Their work is in agreement with that of Honorato (227). Ac-globulin is an accelerator of prothrombin activation. We can thus conclude that the methylxanthines csaue an increase in Ac-globulin concentration and thus indirectly cause prothrombin to be more effective.

It has been shown that caffeine, theophylline and theobromine increase the fibrinogen concentration in the dog and also in the rabbit. In the latter animal some related drugs were also effective (177), namely, xanthine, adenine, uric acid, guanidine and glycocyanine. The rabbit is far more susceptible than the dog to the methylxanthines.

VII. ANTIBIOTICS

The antibiotics have been investigated with respect to alteration in the coagulation mechanism. Ochsner (315) has suggested that the failure to reduce the incidence of thromboembolism in recent years, in spite of improved therapy, may be due to a deleterious effect of the antibiotics. This view has incited additional commentary (152, 315, 437). The available evidence deals primarily with penicillin.

The initial report of Moldavsky *et al.* (361) indicated a lowering of the coagulation and bleeding time following oral and intramuscular penicillin administration. Also non-retractile clots were observed. Macht, in several articles (325-327), reported a reduction in the coagulation time in rabbits, cats, and a few dogs and human beings and emphasized the "thromboplastic properties" of penicillin. Comparing amorphous and crystalline penicillins X, K, G and F, he reported a decreasing effect in that order. The evidence, in fact, for any effect of penicillin G and F is equivocal. The range of dosage used was wide and intramuscular and intravenous routes of administration did not alter the effect. Mosonyi, Palos and Komaromy (365, 366) have supported this *in-vivo* effect of peniclin in reducing the clotting time.

In contrast to the above reports are the investigations of Lewis (295) who followed the coagulation time and, in addition, measured the prothrombin time, capillary bleeding time, clot retraction, platelet count and plasma fibrinogen level. She found no effect after oral doses up to 1,500,000 units per day or single intramuscular doses up to 200,000 units. Similar results were reported after administration of 300,000 units of crystalline penicillin G in oil and beeswax, and there was no change in heparin tolerance curves (148). Hines and Kessler (220) similarly reported no changes in platelet counts or prothrombin times but did find an increased sensitivity to heparin in two patients treated with penicillin. Hypoprothrombinemia has been attributed to penicillin by Lewitus (296) and

Astrup (28), although the latter's three cases appear to have been exceptional and are regarded by the author as manifesting hypersensitivity. In summary of the *in-vivo* effects of penicillin, it appears that the antibiotic causes no major change in the coagulation mechanism within reasonable dose ranges. The possibilities must be reserved that factors not yet measured may be altered and that the various types of penicillin may differ in the responses they cause.

The *in-vitro* effects of penicillin are somewhat clearer. Lewis (295) reported no change up to 500 units per cc. of blood. Fleming and Fish (181) found that concentrations over 3000 units per cc. retarded clot formation and retraction. This inhibiting effect of a very high penicillin concentration has been confirmed by Menghini (347), Mosonyi *et al.* (365) and Strausz (539). Mosonyi (366) suggests that this effect is due to an alteration in fibrinogen, involving —SH groups.

Streptomycin has also been implicated by Macht (325–327) for its "thromboplastic properties," that is, the coagulation time was shortened following administration of the antibiotic. Overman and Wright (390) found a coagulant action under special *in-vitro* conditions. Opposing results (97, 378) demonstrated a retarding effect *in vitro* and no effect *in vivo*. An additional report on intravenous streptomycin administration to rabbits, guinea pigs and cats showed no change in blood coagulation time up to 1 mg. per kg., a decrease in coagulation time after 5 to 40 mg. per kg., and an increase after 50 mg. per kg. (149).

Aureomycin has been investigated by Harned *et al.* (211), Ross *et al.* (459), Lasser *et al.* (317), Shapse and Wright (507) and Herrell (214), who found no significant alteration in coagulation time, prothrombin time, bleeding time, or *in-vitro* heparin tolerance test. Two reports which are contradictory partially disagree with these findings. While agreeing that the prothrombin times are not appreciably changed, Macht and Farkas (328) maintain that the coagulation time is markedly shortened; Galt and Hunter (189) maintain that it is prolonged.

Unfortunately our knowledge of the effects produced by antibiotics on the coagulation process has progressed very little. Most of the reports in favor of an effect do not convince the critical reader. It is plausible that the antibiotics may alter the clotting mechanism, but specifically which mechanism is involved? The matter warrants more thorough investigation.

VIII. PROTAMINES

The action of protamines on the blood coagulation mechanism is complex and it is perhaps profitable to discuss their nature first. In biological materials they are associated with nucleic acids (283) as salt-like compounds. As prepared in the chemical laboratory these proteins have strongly basic properties due to their high arginine content. A preparation of salmine, derived from salmon sperm, contains 58.9 per cent arginine (163). The normal pH of the blood is below the isoelectric point of protamine, and thus the protamines have a charge opposite to that of the plasma proteins themselves.

In 1900 Thompson (562) studied several protamine preparations and found that their intravenous administration may cause a drop in blood pressure, prolongation of the clotting time of the blood, and a reduction in the leucocyte count. After high doses death resulted. In later work Jaques (246) showed that the fall in blood pressure was due to dilatation in the arterioles of muscles. He also found that salmine added to blood in silicone-treated containers caused thrombocytopenia but not leucopenia. Attempts to produce local reactions in the skin at the site of intracutaneous injection were negative. It was also not possible to produce hypersensitivity to protamine in the guinea pig (276).

Shelly, Hodgkins and Visscher (508) studied the toxicity of protamine in the guinea pig and the rat; administration by the intravenous route produced death at a dose level of 6 to 12 mg. per 100 gm. Intraperitoneal injection delayed the lethal effect. When protamine was added to whole blood in the test tube, the cells of the human being, dog, cat and rat were agglutinated. Perfusion of blood through isolated organs was blocked by protamine probably because of embolic vascular phenomena. Other investigations (581) showed the median lethal subcutaneous dose in mice and rabbits to be 200 to 300 mg. per kg. Other untoward effects include respiratory distress, muscular rigidity, marked malaise, polyuria, hyperglycemia, and hemorrhages in the thyroid, lungs and kidneys. Oxygen consumption of minced rat liver is reduced by protamine (446).

The addition of sufficient protamine to blood plasma causes fibrinogen to precipitate (376). This precipitate is soluble in 3 per cent sodium chloride solution. Much of the fibrinogen lyses after incubation of the precipitate. This is probably due to the fact that the precipitate contains, besides fibrinogen, practically all the profibrinolysin of the plasma (203). The precipitation of fibrinogen from human plasma by protamine is also a reversible process for another reason (162). Protaminase, an enzyme present in the plasma of a number of animals, will digest the precipitate. Protamine is also thought to act on prothrombin in test tube experiments (168) but apparently this view has not been put to a test by the use of purified prothrombin. Phosphatides, such as cephalin, form water-insoluble salts with protamine (100). In this manner a lipoprotein such as thromboplastin would be inactived by protamine; Tocantins (572) was able to show that an appropriate amount of protamine could be added to plasma to give an effect which seemed antithromboplastic. He also found that protamine, in certain concentrations, exaggerates the antithromboplastic and anticephalin activity of hemophilic plasma, but not to the same degree as that of normal plasma.

The work of Chargaff and Olson (102) showed that an anticoagulant such as heparin can be inactivated *in vivo* with protamine. This is owing to the combination of the basic protein with the acidic heparin to form a stable salt. This reaction has been studied extensively by many others (245). The action of protamine sulfate, when given intravenously to the heparinized patient or animal, returns the clotting time to normal almost immediately. For use in man protamine is freed of pyrogens (262). The practical use of protamine in the neutralization of heparin developed quite rapidly, despite the natural hesitation stemming from the available knowledge concerning its toxicity (51, 125, 251, 261, 407, 444). The material is given slowly by the intravenous route. A typical commercial preparation consists of a water-clear, one per cent solution of salmine sulfate. One mg. of standard heparin (Connaught Laboratories) is neutralized by 1.28 mg. of protamine, but the figures in the literature for neutralizing a given quantity of heparin *in vivo* are not consistent, probably because heparin itself shows variations in composition. Jorpes, in pioneer work, for example, states that 60 mg. will neutralize 100 mg. of heparin *in vivo* in the human being and that 40 to 50 mg. will neutralize 75 mg. of heparin. The quantity of protamine required for neutralization in the heparinized patient diminishes rapidly with the elapse of time after the prior administration of heparin. If administered immediately after heparin, the amount of protamine required is approximately mg. per mg. that of the amount of heparin, whereas 30 minutes later often less than one half the dose of protamine is sufficient to neutralize the effect of heparin (6a, 6b). The duration of the antiheparin action of protamine sulfate is about two hours. The last traces of heparin are removed very slowly (548).

The quantity of protamine which may be given safely and with best results would presumably depend upon the amount of anticoagulant requiring neutralization. Consequently, and for lack of extensive experience, only examples can be cited. Allen and associates (10) have given 150 mg. intravenously over a period of several hours as the initial dose. In addition 50 mg. were given intramuscularly every four to six hours. Occasionally pain at the site of intramuscular injection has been reported. In cases of leukemia Hill (216) has administered as much as 250 mg. dissolved in fresh blood.

A protamine titration method has been devised for a rough estimate of heparin or heparin-like anticoagulants in the blood. This becomes of interest in conditions such as total body exposure to ionizing radiation (7, 11, 12, 598), the bleeding tendency of thrombocytopenic purpura (9, 408) and the leukemias (573), where heparin or heparin-like materials might be involved. The therapeutic use of protamine, while advocated by some, would seem to require further study, for the results of others have been disappointing. The above named conditions seem to be accompanied by an increase in plasma concentration of heparin or of compounds not easily distinguished from heparin. By contrast normal plasma apparently is almost free of heparin.

IX. TOLUIDINE BLUE

This dye was used by Jorpes and associates (257) to locate the physiological occurrence of heparin in the mast cells. Toluidine blue (dimethyl-orthomethylthionin) shows only slight metachromasia with heparin test tube experiments (250). Some samples of the dye, usually old, show considerable metachromasia. A shift in the absorption spectrum of toluidine blue occurs, and in this respect it is then indistinguishable from azure A (250). Variations in the dye from one sample to another have apparently been encountered quite frequently. Like proteins the dye combines with heparin to form a stable compound. Quantitative measurements show that the reaction follows the mass law (245).

The properties of toluidine blue make it of interest in blood coagulation problems in much the same manner as described for protamine above (7, 9, 10, 12, 225, 230, 250, 407, 408). Protamine will rapidly neutralize an overdose of intravenously injected heparin. Toluidine blue is useless in this respect because of its slow action in neutralizing heparin. On the other hand, the antiheparin effect of toluidine blue, even though slow, can be detected for nearly 24 hours after an initial injection of 4 mg. per kg. in dogs. Allen and associates (9) state that the dye, dissolved as a one per cent solution in 250 to 500 cc. of sodium chloride solution and sterilized by filtration, is well tolerated by human beings in the dose range 1 to 4 mg. per kg. The solution was given slowly intravenously. The toxicity of toluidine blue has apparently not been studied extensively. In dogs but not in man it is strongly hemolytic and causes leucocytosis and thrombocytosis. In man the urine and stool become highly colored and remain so for 36 to 48 hours after a single intravenous dose of 2.0 to 2.5 mg. per kg. The dye is not active when given by mouth (9).

A curious and as yet ill-defined clotting disturbance occurs in patients with certain bleeding disorders associated with, if not caused by, a number of pathologic states. This disordered clotting mechanism is characterized by a prolonged clotting time, a slow rate of fibrin formation, an increased "protamine titration" and clinical evidence of abnormal bleeding (6a, 6b). In some instances, evidence of a clotting inhibitor can be detected. In some respects this inhibitor resembles heparin but in other respects it appears singularly different, with the result that no conclusion as to its nature can be reached at present. The evidence which suggests its heparin-like nature is based solely on the fact that bleeding in many of the patients with the above described syndrome improves after the intravenous administration of toluidine blue, 4 to 5 mg. per kg., for two to four days. The syndrome has been observed in certain cases of menorrhagia, upon estrogen withdrawal in man and dog, in some patients with leukemia, in some patients and dogs exposed to lethal dosages of ionizing irradiation, and as a complication of aminopterin therapy. Some of these conditions are associated with thrombocytopenia, but platelet deficiency alone does not appear responsible for the particular pattern of events described. While toluidine blue is a useful agent in properly selected patients in aiding the control of bleeding, its mode of action in these instances is not known.

X. THE CARDIAC GLYCOSIDES

It is especially difficult to organize the conflicting literature on the effect of digitalis on blood coagulation. The main trend seems to indicate, almost without exception, that there is an accelerating effect of digitalis on blood coagulation when the rabbit is used as the experimental animal. The same is apparently true of the effect of digitalis in the cat, but the experiments with this particular species are limited. This applies also to the dog. With regard to the human being, it is difficult to escape the conclusion that the clinical evidence indicates a predisposition towards thrombosis when digitalis is used. Laboratory work done on human blood is, however, conflicting. There are some trends toward negative results when the criterion of measurement is the prothrombin time or whole blood clotting time. Evidence of hypercoagulability seems to be obtained when extreme care is taken with the technic of determining whole blood clotting time. These generalizations will be examined in more detail.

Apparently the first observation was that of Tanaka (557) who showed that strophanthin accelerates the coagulability of rabbit blood. In work by Field *et al.* (177), lanatoside C and digitoxin administered to rats and rabbits had an accelerating effect on coagulation. There was, however, no effect on the prothrombin time of whole or diluted plasma and there was no antagonistic effect on dicumarol. In another study tincture of digitalis (5 ml. per kg.), digitoxin (0.5 mg. per kg.) and strophanthin (0.1 mg. per kg.) injected into rabbits accelerated the coagulation time of the plasma. It was suggested that the accelerating effect of these drugs is due to the mobilization of prothrombin from the liver because hepatectomy in dogs prevents the effect (542). In another report of the reduction of the coagulation time of rabbits' blood by digitalis, the number of animals was small and the results were not especially striking (603). In the rabbit and the cat digitalis was found to have a pronounced effect in decreasing the coagulation time of the blood (424), and it is recorded by Honorato and Lopetegui (229) that in experiments with rabbits digitalin has a definite effect on blood coagulation. They also record that ouabain, *Digitalis lanata* glycosides, cholic acid or coumarone shortened the prothrombin time as determined on diluted plasma. Honorato and Gomez (228) produced experimental thrombosis in rabbits very readily after pretreatment with digitalis glycosides. A small dose of dicumarol prevented this quite readily. Honorato and associates believe that the decreased prothrombin time observed with diluted plasma may be explained on the basis that the plasmatic co-factor of thromboplastin is increased in concentration. It has already been suggested above that Ac-globulin and the plasmatic co-factor of thromboplastin may be one and the same substance. Consequently it may be that an increase in Ac-globulin concentration of rabbit plasma is caused by the glycosides of the digitalis series.

In experiments in cats, digitalis seemed to shorten the coagulation time of the blood (323). Other experiments by Macht tend toward the same conclusions (324, 326).

Observations on human beings are equivocal. In answer to the title of an article, "Is digitalis indicated in mycocardial infarction?", it is stated that digitalis administered alone for congestive heart failure associated with auricular fibrillation and myocardial infarction would seem to be contraindicated (16). In another study (424) therapeutic doses of digitalis in patients with complicated cardiac decompensation did not significantly affect the coagulation time of blood but it was suggested that, in cases of cardiac decompensation complicated by devitalized tissue due to infarction or trauma, the administration of digitalis may increase the danger of thrombus formation and embolism. It was suggested that this is due to the release by digitalis of an intracellular clot promoting factor—perhaps thromboplastin. Peters, Guyther and Brambel (414) found that digitalization for congestive heart failure in patients with coronary thrombosis increases the incidence of thromboembolic complications and that the hazard could be nullified by dicumarolization of such patients. de Takats *et al.* (554)

point out that it has always been a source of speculation why patients whose auricles have fibrillated for many years suddenly begin to throw emboli, and they state that no attention has been paid to the coagulation system of the blood and that digitalis is received by many such patients. They found heparin to be less effective in the presence of digitalis and while, as they say, there are no data as to the mechanism it must mean that somewhere in the coagulation system digitalis opposes heparin. They also cite a case in which digitalis showed an antagonistic action toward dicumarol. Additional comments by other investigators urge that more careful consideration be given to the possible effects of digitalis on blood clotting (237).

Pere (411) reviewed the literature extensively and made a thorough attempt to ascertain the nature of the influence of digitalis on blood coagulation in studies in human patients. Like many others he investigated the validity of the whole blood coagulation time in man and included a study of the influence of CO_2 and O_2 tension and of venous stasis. The absolute values of blood coagulation time may differ from one person to another, but significant information can be obtained when the determinations are all made by the same person and with careful technic. This idea would seem to be substantiated by those who have developed extraordinary technical skill. Of course, it is another matter when the species being studied has a very short coagulation time. Digitalis shortened the coagulation time in patients with congestive heart failure. On the other hand this did not occur in normal healthy control subjects. Strophanthin shortened the coagulation time in patients with congestive heart failure more markedly than did digitalis; in control subjects no shortening was observed. The mercurial diuretic, novurit (trimethyl-cyclopentane-carbonic-acid-allyl-amide-methoxy-carbonic-acid) and 0.05 gm. of theophylline, also reduced the coagulability time greatly in patients with cardiac insufficiency. In general it seemed that all the drugs that cause rapid disappearance of edema accelerated the coagulation time in cases of congestive heart failure. In control subjects only novurit caused an acceleration of coagulation in which case diuresis was associated with the phenomenon. The prothrombin time failed to show any changes after digitalis, strophanthin or novurit, either in control subjects or in patients with congestive heart failure. The same was true for fibrinogen. In patients with congestive heart failure digitalis increased the daily volume of urine on an average of two-fold, strophanthin by nearly three-fold and novurit by about four-fold. It is suggested that the effect of the drugs is, therefore, not specific but due to hemo-concentration. It is further suggested that as hemo-concentration increases tissue fluid is drawn into the circulating blood and that this increases the thromboplastin content of the blood and so shortens coagulation time. These suggestions extend beyond the limits of the observations, for one must question whether thromboplastin enters the circulation in this manner. It is also known from the work of Tocantins that slight dilution of normal blood in test tubes favors coagulation, most likely because dilution places the inhibitors of coagulation at a greater disadvantage than the activators. Certainly Pere's fine work has indicated possibilities not adequately considered previously.

A number of other workers who have studied the effect of digitalis on human beings have come to the general conclusion that there is no significant effect. In one study, ten patients given digitalis showed no significant alteration in the coagulability of the blood (520). Sutton (544) found no change by the Lee-White method, in the heparin tolerance curve or in the prothrombin times in subjects given digitalis. Likewise Cathcart and Blood (99) found no statistically significant changes in either clotting time or prothrombin time following the administration of digitoxin, lanatoside C or digitalis to patients having congestive heart failure or to normal subjects. Levin and Ruskin (293) also came to similar conclusions.

Moses found no change in the clotting time and no change in response to heparin following intravenous administration of digtalis (364). Almost completely negative experiments with dogs are recorded by Ramsey, Pinschmidt and Haag (441) with the exception that there was a decrease in coagulation time under certain conditions of anesthesia (441). In other reports some patients showed a shortening of the coagulation time and in some it was prolonged (237, 556).

XI. ALPHA TOCOPHEROL COMPOUNDS

Naphthotocopherol was synthesized by Tishler and Evans (570) and was found to have both vitamin E and slight vitamin K activity. Its vitamin K activity was ascertained by bioassay in chicks. In a study of inhibition by structural analogs of metabolites, Woolley (617) found that alpha tocopherol quinone produced manifestations in mice related to those seen in tocopherol deficiency. The administration of vitamin K prevented the resorption of the embryos and the excessive vaginal bleeding which accompanied the resorption process. While the hemorrhagic manifestations produced by alpha tocopherol quinone could be reversed by vitamin K, estimations of the prothrombin time were essentially the same as for the controls so that a decrease in prothrombin concentration probably was not one of the factors associated with the hemorrhage. The author (617) points to the structural similarity of alpha tocopherol, alpha tocopherol quinone and vitamin K_1. Descriptions of vitamin E deficiency have usually included mention of hemorrhagic manifestations (336). Except in chicks on a diet which was probably deficient in vitamin E, hyaline, capillary thrombi were found in and about the necrotic areas of the cerebellum. These thrombi were almost invariably found within and about degenerated areas but it was not possible to present proof that the capillary thrombosis was a primary cause of the ensuing necrosis. It is possible that the thrombi were caused by thromboplastin from the degenerating zones (406, 611).

A number of organic chemicals have a powerful inhibitory effect on the thrombin-fibrinogen reaction (170), while others have a potentiating effect. Among these are gum acacia, phenol, *o*-cresol and *p*-cresol (190). Alpha tocopherol phosphate is one of the compounds which inhibits the action of thrombin. This occurs to a certain extent at a concentration in which the tocopherols are found in normal human serum, which is said to be about 1 mg. per cent. This antithrombic effect of alpha tocopherol could be demonstrated *in vitro* and *in vivo*

(628). With purified reagents the inhibition of thrombin activity in the test tube can be reversed by the addition of calcium because calcium forms an insoluble compound with alpha tocopherol phosphate (273). Kay and associates have devised an antithrombin test which is not based on fundamental relationships and is, therefore, an empirical test (275). They present data to show that there is a thrombosing tendency when this test shows low "antithrombin" values. Furthermore, the administration of alpha tocopherol phosphate with calcium gluconate to patients causes the "antithrombin" value to be increased. The group of patients which received alpha tocopherol phosphate with calcium gluconate had a far lower incidence of thrombosis than a comparable group not so treated (274). They therefore advocate the use of this drug to deal with the problems presented by thrombo-embolic phenomena. It is not easy to decide whether the test tube experiments correlate with the clinical observations. At first one would be inclined to assume a direct connection but *the evidence is incomplete*. The evidence presented by Kay and associates has not been met with much enthusiasm (63) and it remains to be seen whether the clinical conclusions (274) can be confirmed. Overman (63) tried several antithrombin tests and could find no response with various doses of alpha tocopherol in animal experiments.

In another study (272) alpha tocopherol phosphate and inositol phosphatide were shown to have antithromboplastic activity. The tests consisted of producing generalized thrombosis in mice by injection of thromboplastin. After thromboplastin was first incubated with monosidium alpha tocopherol phosphate or inositol phosphatide the mixture could be injected and the clotting effect of thromboplastin was not observed. Under certain conditions *in-vivo* experiments were also successful in that large doses furnished protection against thromboplastin. Fibrinogen counteracts the effects of each of these substances in test tube experiments.

XII. HEPARIN AND RELATED SUBSTANCES

The story of heparin has been summarized in a monograph by Jorpes (257) and it does not seem necessary to review historical aspects such as its discovery, isolation, industrial production, and role in the development of anticoagulant therapy. It does seem appropriate, however, to review recent literature and attempt to cover the pertinent points concerning its chemical nature, mode of action, rationale for methods of administration, and heparin substitutes.

Chemical nature. The commercial products which are available are obtained from animal tissues. In these tissues the anticoagulant is located in the mast cells (257, 606) found mainly in the connective tissue near capillaries and in the walls of blood vessels. The heparin material is found with the amorphous microsome material occupying the "intergranular" cytoplasm (264, 547). From here the native material has been obtained in the form of a heparin-lipid-protein complex. The protein part has an amino acid pattern similar to that of the histones (547). Since heparin as such has not been found in appreciable quantity in the blood it may be that the lipid-protein complex is all that remains when and if the whole native complex is released into the blood stream. In any case the commercial heparin isolated from lungs or liver is devoid of the lipid-protein complex. It is obtained as crystalline material, either as the barium or sodium salt. It is a mucoitin polysulfuric acid which has a molecular weight of 17,000 (256). The repeating unit in the molecule is a disaccharide and consists of glucosamine (257) and glucuronic acid (612, 614, 615) joined through chemical linkage and containing the sulfuric acid as ester. Alternative structures for the general architecture of the molecule are proposed (615). Mild acidity causes the heparin molecule to lose its anticoagulant activity. The rate of loss in activity is proportional to the rate of appearance of a free amino group in the molecule. It is believed that its nitrogen linkage is essential for its biological activity (613). Heparin has a strong negative charge and when added to plasma a new electrophoretic component, the C component, appears and migrates with a mobility between that of heparin and albumin (104). It may be that all heparin preparations are more or less inhomogeneous in that they may possess one, two or three sulfate groups for each disaccharide unit (260).

A unit of heparin is defined as 1/130 mg. of a potent sodium salt of heparin supplied to the National Institute of Medical Research, London, by Best and associates of Toronto. It is the Provisional International Standard and is equivalent to one U.S.P. (XIV) unit. In the United States commercial preparations are adjusted to a potency of 100 units per mg. In Sweden it is standardized to contain 80 units per mg.

Mechanism of action. Heparin is effective at two points in the chemical mechanism of blood coagulation. Firstly it blocks the activation of prothrombin. Actual measurements show that heparinized blood contains its full quota of prothrombin. To block the activation of this prothrombin a plasma cofactor is required, for heparin will not block the activation of purified prothrombin (17, 81, 82, 429). The nature of the co-factor is not known, except that it is probably a protein. Secondly, heparin has an effect as an antithrombin. When added to plasma heparin causes plasma to develop powerful antithrombin properties (431). When heparin is mixed with purified thrombin this powerful inhibition is not observed and it is, therefore, believed that plasma furnishes a co-factor which acts in conjunction with heparin to give the antithrombin effect. It could be that this co-factor is the C component (104) or the protein-lipid complex associated with heparin in the native state (457) or that these two are the same, but the answer is not known. It has been shown, however, that this antithrombin effect of heparin does not involve destruction of thrombin (20, 278). The mechanism is apparently an interference with the ability of thrombin to clot fibrinogen. Heparin thus acts as an anticoagulant whose effect is superimposed on the blood clotting mechanism whereas dicumarol acts on physiological mechanisms to interfere with the production of prothrombin.

From the work of Copley and Robb (120, 121) it became known that heparin affects the platelets. When added to dog blood *in vitro* it produces a platelet decrease; *in vivo* this same effect is transitory and there may be a subsequent increase above the original. In mice single or repeated subcutaneous injections did not result in a significant change in platelet count. Fidlar and Jaques (171) also found a brief thrombocytopenia in dogs and man, and the fall recurred with repeated intravenous injections. There was some variation from one heparin lot to another. In another study it is concluded that the agglutination of platelets is of little moment in the use of the drug (439), with the possible exception that thrombin might cause platelets to agglutinate, in which case heparin would be an agent to prevent agglutination indirectly via the mechanisms described above, namely, by blocking prothrombin activation and by acting as antithrombin.

In small amounts heparin accelerates blood coagulation (154, 178). Thereafter, with higher concentrations, the effects of the above described mechanisms are observed. Upon intravenous injection of heparin, the clotting time is prolonged in proportion to the dose (53, 249). A linear relation is found between dose and the logarithm of the clotting time obtained. With moderate doses five to ten minutes are required for the clotting time to reach a maximum and thereafter the heparin effect becomes dissipated in a few hours, the duration of action being proportional to the dose (53, 244). The drug is probably inactivated by an enzyme. Also its excretion by the kidney has been considered. This route of elimination may not be important (52) for the excretion by the kidney may be limited (122, 257). The excreted material may be a derivative of heparin, called uroheparin (53). It is said to be far less active than heparin itself. In one experiment Jaques and Ricker (249) found no difference in the rate of disappearance of heparin in normal and nephrectomized dogs. On the other hand, in rabbits 25 to 50 per cent of injected heparin was excreted in the urine by glomerular filtration and tubular secretion (421). Deposits were found in the kidney, liver, spleen and lung of the rabbits. Also carinamide (4′-carboxyphenylmethanesulfonanilide), which affects certain renal transport mechanisms responsible for tubular secretion, has been reported to influence the duration of heparin action in man (341). From the practical standpoint it was suggested that the use of carinamide might reduce the quantity of heparin required by 50 per cent (341).

Administration. Heparin is not effective by oral administration. The various methods of administration have taken into account its fundamental pharmacological properties described above. Large doses are not toxic, nor do they affect wound healing (47, 258), and the only danger is that of hemorrhage. The rapid neutralization of heparin action by protamine or toluidine blue, as described below, provides a valuable safety measure. The possible danger of hemorrhage has prompted the use of minimal dosage, but Jorpes (257) has emphasized the need for quantities adequate to prevent thrombosis. The intravenous drip method employed by the pioneering Toronto group attempted to match the slow disappearance of heparin. This method entailed inconveniences and involved large fluid volumes (251, 372). In Sweden (257) intermittent intravenous doses were given, for example, 50 + 50 + 50 + 100 mg. or 75 + 75 + 75 + 125 mg. per day at 8, 12, 16 and 20 hours, respectively. For details the reader should consult Jorpes (258, 259). Strong aqueous solutions have been given by the intramuscular route (533, 587). A repository form of heparin has been used and consists in purified heparin compounded with gelatin-dextrose-acetic acid (450). It caused pain at the site of injection, but this objectionable feature has been eliminated by appropriate adjustment of pH (312). It prolongs the action of heparin effectively (156, 198, 202, 223, 311, 313, 314, 368). The disadvantage of unpredictability has been recorded, but as an average the full effect of the drug is observed at five hours, high effectiveness for the next 20 hours, and significant elevation of the coagulation time for 50 hours. With the addition of vasoconstrictors such as epinephrine and ephedrine the coagulation time is prolonged by the eighth hour, remains at that high level for about 40 hours, and returns to normal at about 70 hours. The use of heparin is widely discussed in the literature and some additional practical views not previously cited are given (41, 281, 309, 333, 334, 452, 552).

Heparin substitutes. The fact that commercial heparin of animal tissue origin is a disaccharide containing sulfate suggested the possibility of making synthetic polysaccharide sulfuric acids. Esters of cellulose, chitin, pectic acid, glycogen, starch, gum arabic and yeast nucleic acid were prepared (22, 23, 24, 37, 48, 49, 270). They were all found to possess heparin-like activity but were far less potent than heparin. Pharmacological studies (26, 418, 419, 420) showed the cellulose and starch sulfuric acid esters to be the most toxic and chitin the least (26); the chitin material had the highest anticoagulant potency (419). The main difficulty encountered was platelet agglutination after intravenous injection (418, 420). Cellulose and starch sulfuric acid esters precipitate fibrinogen and clump the platelets with the result that emboli are produced and later infarcts develop in the lung and kidney (420).

The results with another synthetic compound were far more encouraging. A polysulfuric ester of polyanhydromanuronic acid, called Paritol, has an intravenous LD_{50} for mice of 1898 mg. per kg. as compared with 1500 to 2000 mg. per kg. for heparin. Its anticoagulant potency is about $\frac{1}{7}$ that of heparin (525, 526), but the duration of action is prolonged as compared with heparin. About 30 per cent of an intravenous dose is recovered in the urine, but its action is not prolonged by bilateral nephrectomy, splenectomy, hepatectomy, injections into the bone marrow, or blockade of the reticuloendothelial system with India ink. Sorenson and Wright (525) reported evidence of satisfactory maintenance of anticoagulant therapy with Paritol. Three of 35 patients showed reactions. Two of these reactions were mild and the other was severe; however, the authors feel that further studies are definitely indicated.

Bartholomew and Barker (38) found, in a series of ten subjects, that protamine sulfate neutralizes the effect of Paritol. For example, the coagulation time of whole venous blood which had been markedly prolonged by the administration of 300 mg. of Paritol returned rather rapidly to normal when 200 mg. of protamine sulfate were given intravenously within one hour after the Paritol. Similar effects were noted with 100 mg. given four hours or 50 mg. given eight hours after the administration of Paritol.

XIII. UNRECOGNIZED NUTRITIONAL FACTORS

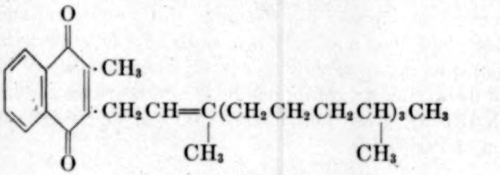
Warner and Owen (595) found that patients with Addisonian pernicious anemia in relapse have a prothrombin level between 40 and 65 per cent of normal. This hypoprothrombinemia is not rectified by large doses of vitamin K but there is a prompt response when specific liver therapy is instituted (595). In further pursuit of this problem Owren (401) found that crystalline vitamin B_{12} or folic acid or both may cause the red blood cell count to return to normal, but the hypoprothrombinemia remains in the range of 60 to 90 per cent of normal. It was also found that the macrocytes are flatter, increased in diameter and more resistant to hemolysis in hypotonic saline solutions. On the latter basis the macrocytes could be separated from the other cells, and it was found that their hemoglobin is of the fetal type as shown by the rate of alkali denaturation. A substance found in crude liver extract returns these aberrations as well as the hypoprothrombinemia to normal. The substance in liver may correct the hypoprothrombinemia and fetal type hemoglobin without altering the red blood cell count. Apparently the liver factor is generally concerned with protein metabolism and the hypoprothrombinemia is only one of several ways to detect the deficiency.

Folic acid is also concerned with one of the proteins of the clotting mechanism. Macrocytic anemia in chicks, resulting from folic acid deficiency, is accompanied by an increase in plasma antifibrinolysin activity. Crystalline folic acid prevents this marked increase (204).

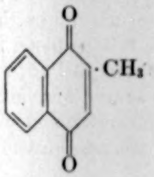
XIV. VITAMIN K

Previous reviews (18, 78, 88, 92, 133, 147, 303, 335, 432, 461, 594) cover most of the field up to present times. Thayer (558) asserts that over 600 papers have been written on the effectiveness of vitamin K in preventing hemorrhages in the new born and it is hardly appropriate to take up this subject or the others covered by the above references. However, at this time it seems appropriate to recall the basic facts and to discuss the relationship of vitamin K to hypervitaminosis A, to hyperprothrombinemia and to antagonistic drugs.

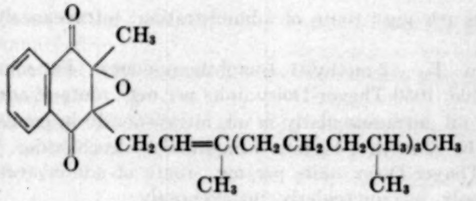
Vitamin K deficiency causes a bleeding tendency because vitamin K is necessary for the synthesis of prothrombin by the liver (132). This mechanism requires a normal functioning liver and in fact a vitamin K tolerance test (578) is a useful liver function test. The vitamin is not a part of the prothrombin molecule itself (495) for it cannot be found in purified prothrombin preparations. There are many compounds possessing K activity and most of them are naphthoquinones. Some of those most commonly available are the following:



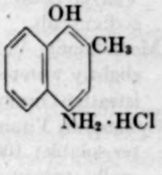
(1)



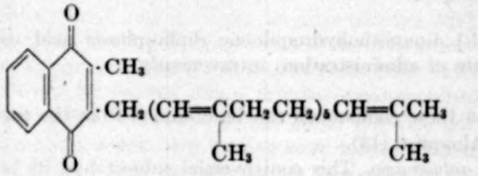
(5)


O
CH₃
O
CH₂CH=C(CH₂CH₂CH₂CH₃)₃CH₃
O
CH₃
CH₃

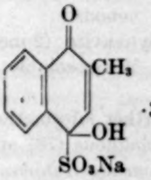
(2)


OH
CH₃
NH₂·HCl

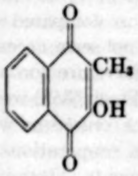
(6)


O
·CH₃
·CH₂(CH=CCH₂CH₂)₅CH=CCH₃
O
CH₃
CH₃

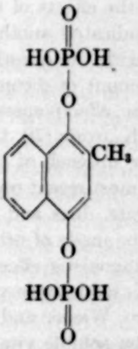
(3)


O
CH₃
·3H₂O
OH
SO₃Na

(7)



(4)


O
HOPOH
O
CH₃
O
HOPOH
O

(8)

(1) **Vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone). Fat-soluble, natural vitamin; melting point, −20°C; 1000 Thayer-Doisy units per mg.; route of administration: orally, intramuscularly in oil, intravenously in emulsion.**

(2) **Vitamin K_1 Oxide (2-methyl-3-phytyl-1,4-naphthoquinone-2,3 oxide). Fat-soluble; route of administration: intravenously in emulsion.**

(3) **Vitamin K_2 (2-methyl-3-difarnesyl-1,4-naphthoquinone). Fat-soluble, natural vitamin; melting point, 53.5–54.5°C; 600 Thayer-Doisy units per mg.**

(4) **Phthiocol; Vitamin K_3 (2-methyl-3-hydroxy-1,4-naphthoquinone). Slightly water-soluble; sodium salt is water-soluble; melting point, 173–174°C; 2**

Thayer-Doisy units per mg.; route of administration: intravenously as sodium salt.

(5) Menadione; Vitamin K_3 (2-methyl-1,4-naphthoquinone). Fat-soluble; slightly water-soluble; 1050 Thayer-Doisy units per mg.; route of administration: orally in oil, intramuscularly in oil, intravenously in emulsion.

(6) Synkamin; Vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride). Water-soluble; 1000 Thayer-Doisy units per mg.; route of administration: orally, subcutaneously, intramuscularly, intravenously.

(7) Menadione sodium bisulfite. Water-soluble; route of administration: intravenously.

(8) Synkavite (2-methyl-1,4-naphthohydroquinone diphosphoric acid ester). Water-soluble; route of administration: intravenously.

Further information on these compounds can be obtained from the reviews of Brinkhous (78) and Almquist (13).

Dicumarol-Vitamin K antagonism. This controversial subject had its beginning in an erroneous report by Quick (430) stating that vitamin K does not counteract the effects of toxic sweet clover hay in rabbits (430). Link's (303) work soon indicated another conclusion and clinical as well as experimental reports dealt with the questions of adequate dosage of the vitamin as compared with the amount of dicumarol being given, sensitivity of the patient to dicumarol, and the effectiveness of water-soluble forms of vitamin K as compared with K_1 or K_1 oxide (70, 115, 126, 136, 241, 318, 438, 504). It does not seem necessary to take up each of these studies individually for the main facts are contained in the most recent reports (62, 358, 506). Miller, Harvey and Finch (358) worked with rats, dogs and man and made an attempt to overcome criticisms which could be made of other reports. The water-soluble vitamin K preparations had no antagonistic effect, under the conditions of their experiments. Vitamin K_1 and K_1 oxide were very effective in decreasing the prothrombin time. To this Shapiro, Wiener and Simson (506) add the observation that a single large dose of water-soluble vitamin K may be adequate to correct hypoprothrombinemia in man when there are relatively low concentrations of dicumarol in the plasma for a short duration of time. Overman, Sorensen and Wright (393) point out that the conclusions of Miller, Harvey and Finch (358) tend to be misleading. Under the conditions of their (358) experiments vitamin K and dicumarol were administered simultaneously, a condition which should not occur in the clinical use of dicumarol. Under clinical conditions Overman, Sorensen and Wright (393) did find water-soluble vitamin K effective in counteracting excessively prolonged prothrombin times due to dicumarol. Patients with persistently high plasma concentration of dicumarol may require repeated large doses to bring the prothrombin time to a safe level and control its excessive prolongation. Since the water-soluble forms fail under certain conditions and are required in repeated doses it can only be concluded that K_1 and K_1 oxide are comparatively more effective. The word *effective* is used instead of *superior* because the solubility characteristics of the fat-soluble preparations may make them more slowly disposed of than water-soluble forms and thus permit them to exhibit a more sustained effect. They may not necessarily function better in the actual chemical interactions. The massive doses of the vitamin required are stressed in most of the papers.

In hypervitaminosis A the most characteristic lesions are skeletal fractures and hemorrhages, the latter being predominant in adult animals. In rats overdosage of vitamin A is associated with a prolonged prothrombin time, and this effect can be counteracted by the administration of vitamin K (2-methyl-3-phytyl-1,4-naphthoquinone) (299). It was also found possible to use water-soluble forms of vitamin K for the same purpose (588).

Field and Link (173) have reviewed the question whether vitamin K can produce high prothrombin levels in the blood. Their own experiments with the dog, rabbit and rat showed that a definite hyperprothrombinemia can be produced and is readily detected with the use of the 12.5 per cent plasma method for testing prothrombin time. In man large doses of K (menadione) produced similar effects (577). Where the increase was demonstrable, it continued for brief periods only, lasting 24 to 48 hours.

XV. SULFONAMIDES

When fed to rats given a synthetic ration, sulfaguanidine produces a hypoprothrombinemia as measured by the 12.5 per cent plasma method for testing prothrombin time (57). This prothrombin deficiency can be corrected by feeding *p*-aminobenzoic acid, liver extract or vitamin K. The observations are thought to be explained adequately on the basis of bacterial synthesis of vitamin K in the intestinal tract. The *p*-aminobenzoic acid and liver extract probably act by nullifying the effect of sulfaguanidine, and the bacteria of the intestine then flourish and produce vitamin K. Succinylsulfathiazole was fed in the same manner and with the same results. In another study sulfathiazole, fed at a one per cent level in the ration, also caused rats to develop a prothrombin deficiency which could be corrected by vitamin K (71).

Some of the sulfonamides also have a direct effect on blood coagulation. With purified prothrombin preparations certain sulfonamides inhibit while others accelerate the autocatalytic activation of prothrombin in 25 per cent sodium citrate solution (484, 493).

XVI. ADRENOCORTICOTROPIC HORMONE

In a series of patients the whole blood clotting time was found to be shortened as was also a heparin-retarded coagulation time of venous blood (124). In further detailed studies no changes of significance were found in the prothrombin time or in the protamine titration tests. The authors consider the hypercoagulability of the blood caused by ACTH or 11-dehydro-17-hydroxycorticosterone (cortisone) of importance to the patient's health. In another study (518) it was found that the adrenal cortex seems to exert an effect on a number of constituents of the clotting mechanism, and that the response is not the same from one patient to another. From their work it is evident that further studies are required. These authors (518) also cite the work of Dougherty and Dougherty which showed changes in the mast cells of experimental animals receiving 11-dehydro-17-hydroxycorticosterone (cortisone); as discussed elsewhere in this review these cells are concerned with heparin metabolism.

XVII. ANESTHETICS AND RESPIRATORY GASES

That chloroform anesthesia produces injury to the liver of animals has been known for a long time. Whipple and Hurwitz (604) were able to confirm a prior observation and establish the fact that the fibrinogen of plasma practically disappears with the advance of the liver necrosis after chloroform intoxication. The liver repair process is rapid and the blood fibrinogen values may return to normal in approximately a week. In a further extension of this work by Smith, Warner and Brinkhous (516) it was found that plasma prothrombin concentration also decreases after deep chloroform anesthesia. Furthermore, it was possible to show that prothrombin may disappear without a marked drop in fibrinogen levels, thus indicating that the liver is more susceptible to changes influencing prothrombin production than to those affecting fibrinogen production. Oxygen given in excess with the chloroform does not minimize the drop in prothrombin concentration (129). In man chloroform anesthesia also causes a fall in the prothrombin level. That these effects of chloroform are indeed on the liver and do not operate by some other mechanism is substantiated by the work of Uvnäs (580). The occlusion of the arterial blood supply to the liver causes a rapid fall in prothrombin concentration. The occlusion was performed either by ligating the hepatic vessels in cats and rabbits or by making a modified Eck fistula in cats. Partial hepatectomy in rats also causes the prothrombin concentration to drop (593). Acute hepatic damage caused by chloroform intoxication also causes plasma Ac-globulin concentration to decrease (546). Its concentration returns to normal more rapidly than prothrombin. It is thus established that prothrombin concentration decreases more readily than fibrinogen concentration and the position of Ac-globulin with respect to these two still needs to be established. Owren (400) finds that, in the course of development of a liver disease, the plasma prothrombin level falls sooner and more severely than the factor V (Ac-globulin) level.

Attention has been directed toward the question whether common anesthetic agents cause sufficient liver injury to produce a postoperative fall in prothrombin concentration. Apparently the changes which commonly gain the physician's attention can be ascribed to indirect effects in patients with obstructive jaundice or biliary fistulas (129), for neither ether nor cyclopropane causes a drop in prothrombin concentration. In all but one patient no prothrombin reduction appeared with the following: diethyl ether, divinyl ether, ethylene-oxygen, nitrous oxide-oxygen and tribromoethanol, and procaine and nupercaine spinal anesthesia (8). In another study no changes in prothrombin were found with local anesthesia; but with nitrous oxide plus ether there was a tendency for the prothrombin index to drop and this became more marked on the fourth day after operation. The figures were statistically significant (68).

Another possibility is an increase in prothrombin concentration. Levy and Conroy (294) made careful prothrombin time studies when the patients entered the hospital, before ether anesthesia and during deep anesthesia. At this latter time the presumptive test for prothrombin showed an increase in concentration. This did not occur with spinal anesthesia. Another observer records a slight reduction in coagulation time of the whole blood (482), and the same was found by Searls who also found an increase in platelet count (481). Most of the evidence seems to indicate hypercoagulability of the blood, but this is not a profound change and there is no way of knowing from the present literature which constituent(s) of the blood is responsible. Perhaps shifts in blood volume can account for the differences. For example, it has been known for a long time that post-hemorrhagic blood is hypercoagulable, a constant finding even if the adrenals or intestines are excluded in the experiments (201). Perhaps the dilution of the hemorrhagic blood alters the balance between activators and inhibitors of prothrombin so as to favor the activators. This possibility was beautifully demonstrated by Tocantins (574) and such shifts could easily be the basis for the effect of ether anesthesia. Mendenhall (346) also found that ether anesthesia lowers the clotting time of whole blood and showed that the effect is mediated wholly through the action upon the adrenal glands, because the effect was not obtained after adrenalectomy.

It is stated that pentobarbital sodium lowers the clotting power of the blood (294), and in another report that the clotting time is shortened (29). In experiments with pigeons and cats (371) the coagulation time of the blood was shortened during the first few hours of barbital anesthesia, and returned to normal between 20 and 48 hours. This change went parallel with the blood sugar values and it is suggested that the adrenal glands are involved.

Pere (411) has reviewed the literature concerning the effect of the respiratory gases O_2 and CO_2. Apparently a decrease in CO_2 tension favors clotting and an increase in CO_2 tension has the opposite effect. In a special kind of "antithrombin test" arterial blood was found to inactivate thrombin more readily than venous blood; it is therefore reasoned that arterial blood clots less effectively (405) than venous blood with its heavier charge of CO_2. Pere's own experiments on venous stasis showed that clotting was first accelerated and then retarded. Quite evidently these various experiments involve different mechanisms and it is not possible to make a generalization about the effects of CO_2 or O_2. In asphyxia experiments with dogs there were no significant differences in the clotting times of blood specimens removed before, during and after periods of severe asphyxia (422). Some additional experiments on this subject are recorded, but the reviewer has had access only to the abstracts (155, 371, 404, 479). Quastel and Recker (428) look at the problem from another viewpoint. An extract of the muscle of a limb rendered anoxemic by application of a tourniquet brings about the clotting of blood at a much more rapid rate than an extract of normal healthy muscle. When rat tissue slices were allowed to incubate in the Warburg apparatus under aerobic and anaerobic conditions the latter yielded the highest concentration of clot-accelerating material. This may be a factor in shock, but in any case it shows that tissues undergo changes under anaerobic conditions which are concerned with blood coagulation.

XVIII. EPINEPHRINE

In experimental studies of the sugar content and the extravascular coagulation of the blood after administration of epinephrine, Vosburgh and Richards (584) found in every case that the time of coagulation is lessened. The dimunition was equal in some cases to ⅘ of the control coagulation time. They suspected a relationship between blood coagulation and the pancreas. In further pursuit of this problem Cannon and Mendenhall (94) devised a graphic method for recording the coagulation time of whole blood, thus placing more reliance on this method as against ordinary test tube experiments. Epinephrine injected in large doses intravenously and in large doses subcutaneously shortened the coagulation time to ½ or ⅓ the control duration. When the blood was confined anterior to the diaphragm or when the intestine or liver was removed epinephrine in small doses did not cause rapid clotting (93). It was suggested that epinephrine accelerated the clotting process by stimulating the liver to greater activity, by disturbing some factor or factors in coagulation. Stimulation of the splanchnic nerves (95) resulted immediately or after a brief delay in shortening the coagulation time of blood. The period of rapid clotting lasted from 10 to 30 minutes and the effects were less marked with repeated stimulation. The removal of the adrenal gland on one side nullified the effect of splanchnic stimulation on that side whereas stimulation on the other side was still effective. Stimulating the nerves supplying the liver and intestine did not hasten clotting and it was concluded that the adrenal gland is directly involved. In further work Cannon and Mendenhall (96) concluded that emotional excitement is associated with very rapid clotting of blood and that the blood clotting time is prolonged when the splanchnic nerves are cut. They cite the fact that cats differ widely in their responses to excitement in the laboratory. Some, especially young males, become furious; others especially elderly females, take the experiment quite calmly. This difference of attitude was used to test the effects of emotions on blood clotting. Substantially the same ideas are expressed by Selye in discussing the general adaptation syndrome and he cites further original experiments (500). Link also found that epinephrine hastened the restoration of normal prothrombin time when given to animals about to bleed from excessive doses of dicumarol (303). The experimental demonstration of this fact involved care in getting the animals adapted to laboratory surroundings. The work of Bradfield (200) explains the epinephrine effect as being due to an increase in the amount of prothrombin in the circulating blood, but the data presented do not permit the conclusion that prothrombin is the specific substance involved because methods for the quantitative determination of prothrombin were not available at the time his work was done. However, Uvnäs (580) reports that there is an increase in plasma prothrombin levels (prothrombin time) when cats are given intravenous injections of epinephrine and after electrical stimulation of the splanchnic nerve. This could, of course, still involve factors other than prothrombin. In a thorough study of the entire problem with the use of the prothrombin time method with diluted (12.5 per cent) plasma, Wakim, Fink and Chen (586) come to the following conclusion: The effects of different doses of epinephrine administered to dogs or rats intravenously, intramuscularly, and subcutaneously justify the conclusions that the drug produces no significant change in the prothrombin time of diluted plasma. In dogs made hypoprothrombinemic by dicumarol the effects of epinephrine are variable and inconsistent and, therefore, probably insignificant. The fact that this *variability* was encountered would tend to support the general view that stress states are factors to be considered and these were probably not ruled out in the experiments of Wakim, Fink and Chen. At this point it perhaps needs to be recalled, as already mentioned above, that the hypercoagulability of post-hemorrhagic blood is due to causes other than those involving epinephrine.

de Takats devised a test of the clotting mechanism referred to as the heparin tolerance test. It is essentially a simple test wherein a small amount of heparin is injected intravenously and the coagulation times are then determined at ten-minute intervals for about 40 minutes (550, 553). Epinephrine increases the heparin tolerance. In other words, a hypercoagulable effect counteracts the anticoagulant response to heparin (551). By using the same test Moses (364) found no significant or consistent change in five human subjects following the administration of 1 mg. of epinephrine intravenously. Perhaps the fact that the changes were inconsistent is of importance for reasons inferred above. The statement is made by Moses (364) that epinephrine reportedly hastens coagulation of blood but such reports have not been generally confirmed. The facts in the literature are to the contrary.

There are papers which indicate that the effect of epinephrine is due to the activity of certain of its oxidation products produced in the tissues. The chief product is thought to be adrenochrome. The hemostatic effect of this substance is currently being studied (30, 31, 141, 179, 180, 425, 457, 458, 519).

Another effect of epinephrine is concerned with fibrinolysis (56). Epinephrine was injected subcutaneously in human volunteers, blood samples were taken within an hour, and fibrinolysis was allowed to proceed for 24 hours. In general control samples did not lyse whereas the experimental samples lysed extensively. *In vitro*, epinephrine did not lyse the blood, so that the effect must have been mediated via physiological processes and these most likely involved the activator of profibrinolysin in the manner outlined in the introductory remarks to this review. It is quite possible that the fibrinolysis associated with exercise, fear, trauma and some pathological states results indirectly from the stimulation of epinephrine secretion. However, another possibility is that epinephrine is not to be regarded as having a very specific role, for it has been found that parenteral administration of procaine or even sodium chloride solution causes lysis of blood samples (267).

XIX. ACETYLCHOLINE

de Takats (551) expresses the view that there can be no doubt that the adrenergic and cholinergic components of the autonomic nervous system exert an effect on the clotting mechanism. Puharich and Goetzl (424), in a review of the literature, develop the same ideas and even point to the suggestion that a center for the regulation of blood coagulation may exist in the diencephalon. It is suggested (551) that parasympathomimetic drugs such as methacholine and neostigmine tend to constrict the hepatic veins and prevent a steady flow of prothrombin from the liver, and in this way contribute to the hypocoagulability of the blood. de Takats points out that other possibilities must be considered. It seems to the reviewer that more data are required to determine which substances and/or physiological mechanisms are involved. As an example of the complexity of the situation the work of Soulier and Koupernik (528) shows that fibrinolysis takes place regularly in blood withdrawn between the second and tenth minute after acetylcholine-induced shock. This most likely involves the fibrinolysin activation system.

XX. BLOOD COAGULATION COMPONENTS

The advances in our knowledge of blood coagulation have been made, in part, as the result of methods devised for preparing some of the important components in purified form. Heparin is an example, and has already been discussed from the standpoint of its uses and pharmacological action. Some of the others are also of importance and, therefore, need to be discussed.

Thrombin. The methods used for the industrial production of thrombin are essentially the same as those employed in its production for research purposes (21, 113, 114, 369, 483, 489, 491, 492, 494, 495, 543). A unit of thrombin is that amount which will cause one cc. of standardized fibrinogen solution to clot in 15 seconds. Standards are maintained in the United States by the National Institutes of Health, and the World Health Organization anticipates the use of this standard.

Following the introduction of thrombin as a hemostatic agent (496, 597) its extensive use (50, 90, 106, 110, 123, 127, 128, 131, 159, 184–187, 213, 238, 298, 300, 301, 409, 417, 427, 440, 443, 445, 454, 538, 559, 566, 567, 601, 625, 626) required further knowledge concerning its pharmacological properties. It has been found, as one would expect, that the intravenous injection of thrombin causes deposition of fibrin and the many dangerous consequences of such deposition. Apparently the intravenous injection of minute amounts of thrombin causes an increase in the defensive mechanisms of the organism (191) and may shorten the bleeding time (555). This is obviously a dangerous way in which to shorten the bleeding time. Typical responses to the injection of strong thrombin solutions are those reported by Astrup and Volkert (25). Rapid injection into the femoral vein causes most of the animals to die in one to two minutes in convulsions. The animals may survive for a matter of minutes and develop a large embolus in the pulmonary artery. When larger doses are injected intramuscularly or subcutaneously there is no gross response and the animals survive without any apparent serious consequences. The same has been observed in rats (597). In rabbit experiments (25) no response was seen to intraperitoneal injections but massive doses in rats (597) seemed to cause extreme discomfort, inasmuch as the animals rolled and tossed about. On examination of the blood it was found to be partially and in some instances completely defibrinated. Apparently there is absorption of thrombin from the peritoneal cavity when such tremendous quantities are given. This has also been observed by Zucker (63). Injection into such vital organs as the liver may cause sudden death (491). There is a drop in blood pressure when thrombin is given intravenously; this is due to its clotting power, for the effect can be abolished by preliminary heparinization (549).

Under proper conditions and with a certain amount of luck it is possible to administer thrombin intravenously and completely defibrinate the animal (265, 382). Jürgens and Studer (265) completely defibrinated many animals in this manner. They then identified fibrin in the lung, the kidney and the liver. They also found that these fibrin deposits do not remain there very long after the thrombin infusion. Some mechanism for their removal must therefore exist. When the animal is completely defibrinated there is danger of bleeding. It is interesting that a massive dose of thrombin causes changes in the vascular tree which actually favors bleeding. It seems as though thrombin alters the capillary permeability. The above authors also record a tremendous drop in the leucocyte count after thrombin infusion. The active thrombin is neutralized in due course by the normal antithrombin of the plasma. The return of the fibrinogen concentration to normal values takes place over a short period of about three to four hours.

The practical use of thrombin is confined to topical application to bleeding surfaces and the above untoward pharmacological effects are avoided entirely. However, thrombin is a protein and its use, when it is prepared from bovine material, has raised the question whether antigenic properties are of importance. There is no difficulty in demonstrating the antigenicity of thrombin preparations when the appropriate procedures are employed to elicit an antigen-antibody reaction (135). In contrast, the proper medical use of bovine thrombin has in no instance produced an antigenic reaction. In a direct consideration of this question Light (298) used bovine thrombin surgically to control hemostasis during brain operations in monkeys. They did not develop either serum precipitins or any local tissue reaction in the brain (Arthus phenomenon). In 15 patients precipitin tests, done from 10 to 94 days following the neurosurgical use of bovine thrombin as a hemostatic agent, yielded no positive reactions. On the basis of extensive clinical experience Tidrick (105) has also found no undesirable results from the use of thrombin. None of his patients has shown any evidence of absorption of thrombin or of local thrombosis of vessels (567). Even with the use of fibrin-thrombin films in skin transplants (566) there were no reactions observed; moreover, in this work *bovine fibrinogen* was used successfully as a source of fibrinogen for the surface of the transplants.

Prothrombin. The use of prothrombin in experimental work has not been extensive nor has the substance been introduced for practical use. The possibility of restoring prothrombin deficiencies is self-evident but so far it has not been possible to furnish the material in the required quantities. In dogs the hypoprothrombinemia produced by dicumarol could be corrected by the intravenous administration of a prothrombin preparation (343). The normal prothrombin levels of the blood produced by intravenous injection are of short duration. After about 24–48 hours the prothrombin values are again found to be very low, which is in accordance with the general finding that prothrombin disappears from the blood rather rapidly when the stores are not replenished by a normally functioning liver. The experiment of McGinty, Seegers, Pfeiffer and Loew (343) has recently been confirmed in all respects (536).

Streptokinase-streptodornase. The bacterial product, streptokinase, is capable of activating profibrinolysin and has been prepared (105) in combination with streptococcal desoxyribonuclease (streptodornase). In clinical studies (509, 569) intrapleural injections of these substances caused intrapleural fibrinolytic and proteolytic changes, the effects of which were self-limiting after each injection. Toxic manifestations from the injections were limited to transient febrile reactions with general malaise and a local outpouring of leucocytes which lasted for a few days. The use of these substances by Tillett and Sherry (569) has indicated the importance of streptokinase and streptodornase in influencing the course of certain exudative disease.

Thromboplastin. (*a*) *General remarks.* Crude preparations from organs of different species can be prepared easily and some are available commercially as laboratory reagents. Partial purification can be accomplished by taking advantage not only of the usual precipitation methods but also of the high molecular weight which permits differential sedimentation of thromboplastin by high speed centrifugation (103). The thromboplastin which can thus be obtained is not to be confused with other accelerators of coagulation, such as platelet factors (485). Topical application of thromboplastin has been suggested for local hemostasis (118). For this purpose it is wholly dependent for its action upon the available prothrombin of the blood. The value of its subcutaneous or intramuscular administration in traumatic or other hemorrhagic conditions (579) is debatable. The suggestion that thromboplastin be given intravascularly for the treatment of hemophilia, anaphylactic shock or other types of shock (118) can only be regarded with skepticism; this route of administration would appear to be most hazardous according to our present knowledge.

Purely pharmacological studies of intravascular coagulation by injection of "tissue fibrinogen" (tissue extracts) were reported by Wooldridge in 1886 (616); these were extended in the studies of Mellanby (345) who, however, used snake venoms instead of thromboplastin. Hanzlik and Karsner (209) injected lung extract thromboplastin as one of various agents during a study of intravenous therapy in relation to anaphylactoid phenomena. They were not interested in differences between the effects of the intravascular injection of lung extract and those of "the otherwise inert" agents: agar, kaolin, India ink, ricin, or intravenous medications, all of which caused severe reactions in their animals, including death and/or acute pulmonary changes, among which were peribronchial edema, embolism and thrombosis (209).

(*b*) *Experimental pathology and relationship to pregnancy.* The remaining pharmacological studies have been the by-product of experimental pathology. Several of these have been in relation to toxemia of pregnancy (145, 146, 462, 466–477), one was incidental to virus studies (560, 561) and one was in relation to cerebrovascular accidents (221, 608). The toxemia investigations have several times led to the conclusion that the placental "toxin" under consideration was thromboplastin and that its action was that of intravascular coagulation. This was also concluded by the earlier German workers (146, 466, 582) who recognized thrombi in the liver and in other organs. The Japanese workers likewise found that the placental "toxin" of Obata (388) was a tissue kinase of coagulation which resulted in pulmonary and other thrombi and could be promptly lethal. Meanwhile Dieckmann (145) had carried out investigations on the experimental production of liver necrosis, in which he was influenced by the concept of Mills (359) that the tissue factor of coagulation was a "tissue fibrinogen" in the sense of a kind of plasma fibrinogen. More recently, in a series of experiments, Schneider (467–477) concluded that the potent agent of extracts from the placenta and from the decidua is thromboplastin in the modern sense of that term. Intravenous injection resulted in vascular occlusions which had a predilection for the lesser circulation (473–476, 486, 490). These occlusions had a characteristic fibrin structure (473–476, 486, 490) which suggested that they had been built up by a process of "fibrin embolism" (475).

Mice (470) and rabbits (473) are two to three times more sensitive during late pregnancy than normal animals. Pregnant dogs are also more sensitive as shown by the fact that a dose of tissue extract which is not lethal for non-pregnant dogs is promptly lethal late in pregnancy (608).

(c) *Effect on circulation.* In experimental animals, the markedly effective route of administration is intravascular because this permits activation of the blood coagulation mechanism. The result may be prompt death caused by vascular occlusion. Sublethal doses cause a variety of pathological disorders consequent upon intravascular coagulation and these can be varied to some degree by the manner of injection. Whether physiologic disorders of a more elusive nature result from administration by other routes apparently remains to be determined, although some qualitative comparisons have been made (210, 471). The greater portion of the circulating blood becomes solid only if the intravenous dose is enormous. Intravascular injection of large doses may also cause severe reactions without death, but then the blood will no longer clot. This is because the fibrinogen is almost quantitatively deposited from the circulation (188). As a result the animals are temporarily refractory to further injection of the tissue extract (188, 345, 467). If the fibrinogen is restored artificially to the blood *in vitro* (345) or *in vivo* (188) the sensitivity to further additions of thromboplastin is restored. If a large dose of thromboplastin is given slowly or if repeated small doses are given, it is possible to render the animal completely refractory to further injection of even massive doses (188, 467, 471). If the animal is permitted to restore its own fibrinogen, nearly full sensitivity returns within one day or slightly longer (467), and it is probable that the fibrinogen restoration is accomplished in the same time (475) or even in less time.

If the injection is a lethal one, death in coma or convulsions may occur in a very few minutes. If essentially complete defibrination is brought about, this in itself does not result in hemorrhage, nor may there be outwardly visible evidence of the profound disorder of the coagulation mechanism. However, if bleeding is initiated by trauma or by surgery, fatal hemorrhage may ensue because of failure of the hemostatic mechanism (474, 475, 486, 490).

Among the secondary complications, death occasionally occurs after a delay. Rarely, violent premonitory convulsions have been observed in mice within a few minutes or hours (471), presumably as a result of an embolus to a vital area. In dogs, "thrombosis" of the portal circulation has been observed to cause delayed death (471, 608). After repeated injections, neurologic signs and even eventual death may result from massive hemorrhage in vital cerebral areas (608). These effects seem to be rare following single injections and it is to be wondered (476) whether they may be due to a combined effect of focal necrosis from an earlier injection (221) and the stress of one or more later injections (608).

Because of the tendency for immediate fibrin deposition within the lesser arterial circulation following injection of thromboplastin, there may be a resultant tendency for the development of an acute cor pulmonale (476). However, judging from the rarity of delayed deaths, it is unlikely that death is often caused in this manner by a single injection, without an underlying pathologic process in addition. Possibily as a result of fibrin deposition, parenchymal lesions may develop in various organs of surviving animals. An example is liver necrosis which in the mouse is focal in distribution (469) and in the rabbit it is midzonal (473). Degenerative kidney lesions (478) may be a result of fibrin deposition or perhaps they result secondarily from the hemolysis which occurs with extensive intravascular coagulation.

The prompt death that is so readily caused by injection of thromboplastin is probably due to peripheral circulatory failure (472). *In-vivo* microscopic observations (472, 561) show that the circulation ceases although the respiration and the heart beat continues for a short time. This cessation of blood flow also occurs with the sublethal reactions, but in these cases the pulsating circulation is soon reestablished. Microscopic, *in-vivo* observations (472, 561) may reveal a momentary shower of arteriolar emboli before the blood flow stops in the peripheral vasculature. It is possible that the chief site of occlusion is within the lesser circulation, for it is there that fibrin occlusions are found to be widely disseminated upon microscopic examination of the tissue sections. In fact, one group of investigators finds that "thrombi" within the central nervous system are rare, although minute perivascular hemorrhages therein are frequent (608). Hence, the evidence points toward occlusion of the lesser circulation as the cause of death. This view is supported in another way; in the organs of animals recently dead of thromboplastin injection, there is splanchnic pooling of the blood, including massive engorgement of the liver with distention of its capsule (469, 476). Furthermore, the occlusion of the lesser circulation appears to be aggravated by constriction of the pulmonary artery (279). This combined evidence points to the possibility that the minute hemorrhages and necrosis in the central nervous system of survivors (221, 608) may be as much on the basis of anoxia from temporary circulatory failure as on the basis of direct local obstruction by fibrin deposition. Similarly, an apparent failure of cardiac and respiratory centers may be secondarily due to anoxia caused by occlusion of the lesser circulation.

(*d*) *Nature of fibrin deposits*. The fibrin deposits are chiefly intrarterial, are widely disseminated, especially in the lung (473, 474), and are of a characteristic type (473, 474, 486, 490) which suggests the mechanism of their deposition as one of "fibrin embolism" (475). The term *fibrin embolism* means that the deposits are built up by oriented deposits of myriads of fibrin elements from the pulsating circulation (475). These vascular plugs are not mere blood clots such as occur in a test tube. The best display occurs when large doses of thromboplastin are given; the fibrin plugs are massive and dense although still essentially microscopic. They appear as cords of fibrin occluding the pulmonary arteries of all sizes (473–477, 486, 490). The fibrin elements of such occlusions are oriented parallel to the axis of the vessels, and are densely arranged. This is best seen in longitudinal section. There are few erythrocytes, and these are trapped in columns within the fibrin matrix. There are relatively many leucocytes. These give a clue as to the manner of deposition of the fibrin, for some of their nuclei are stretched in the same axis as the fibrin, as though deposited within fibrin masses under tension; this distortion is sometimes so extreme that a single nucleus may extend as a beaded filament beyond the limits of an entire high power field of the microscope. In cross sections, many of these fibrin occlusions have a minute central core of clotted blood, and from this it may be wondered whether, during the process of deposition of the fibrin from the moving stream, the cross-sectional area of flow ultimately became so small and the flow so retarded that coagulation of the remaining central core occurred without differential deposition of fibrin (476).

When large but less concentrated doses of thromboplastin are injected, the fibrin occlusions may be less dense. At the opposite end of the scale, if the dose is small, there may still be fibrin deposition; the deposit is then more loosely arranged and less well oriented, but has within it such an unusually large number of leucocytes as to give the appearance of a white cell thrombus (476). The above results follow upon single injections. In contrast to the characteristic fibrin deposit, another type of deposit has been reported, and demonstrated with excellent color photography (608). It is laminated, and the laminations are of different ages, corresponding probably to the different times of the repeated thromboplastin injections.

Fibrinogen. This protein becomes the fibrin clot in the blood coagulation process. It has been made available by the methods of plasma protein separation of Cohn and associates (112). The preparation contains the protein in concentrated, dry form and is thus stable for long periods. Its usefulness in human cases of afibrinogenemia has been reported (363, 599, 600). A person of average body weight can be expected to have about seven to ten grams of fibrinogen in the circulation. If this is completely depleted the hemostatic mechanism can be restored by returning the fibrinogen artificially. It is, however, not necessary to have complete restoration. In one instance three grams of fibrinogen and 3000 cc. of blood were administered effectively (600). Two to six grams are recommended by Weiner, Reid and Roby (599). It is of interest that afibrinogenemia is of concern in obstetrical practice (134, 363, 402, 475, 599, 600); apparently placenta abruptio or partial placenta abruptio may first cause intravascular coagulation (475) and, when this is sufficiently extensive, the fibrinogen of the plasma may be completely depleted.

XXI. SUMMARY

The evaluation of the pharmacological actions of drugs on blood coagulation requires a fundamental knowledge of the blood clotting mechanism and the use of analytical technics whose limitations are realized. Many of the data from the papers considered in this review were obtained before much of our current fundamental information was available and before the technics which can be employed today had been devised. As a consequence, a number of these papers are devoid of definitive merit. Therefore, an attempt was made to interpret the vast amount of conflicting data in terms of modern concepts, and to indicate the need and the possibilities for further studies of the influence of drugs on blood coagulation and related phenomena.

3,3'-Methylenebis (4-hydroxycoumarin) or dicumarol decreases the prothrombin concentration of plasma. Its action is probably in the liver, and it may be that the drug itself rather than one of its metabolic derivatives causes the effect. Dicumarol produces a transitory drop in plasma Ac-globulin concentration, and probably affects the plasma concentration of one or more of the new blood coagulation factors which have recently been discovered. Dicumarol is secreted in milk but perhaps not in sufficient quantity to be of therapeutic significance. There is no liver damage caused by the drug. In this respect, and apparently in all other respects also, the metabolic action is completely reversible. Massive doses of vitamin K can greatly accelerate recovery from the effects of dicumarol. This generalization includes the action of both the water-soluble and the fat-soluble forms of the vitamin. The intravenous administration of purified prothrombin restores the prothrombin concentration of the blood to the normal level.

Several compounds related to dicumarol produce essentially the same qualitative effects as produced by dicumarol itself. Quantitative differences are, however, found. These pertain to the rapidity with which the prothrombin concentration falls when the compound is administered and the rapidity with which the prothrombin concentration returns to normal when the compound is withdrawn. Species differences in response to compounds related to dicumarol have been described; for example, rats are especially sensitive to warfarin and, therefore, it is a valuable rodenticide.

In large quantities the salicylates may produce a hypoprothrombinemia, and this effect is quite readily prevented or reversed by vitamin K. The methylxanthines, given in relatively large doses, cause the blood to be hypercoagulable. One of the contributing factors in this effect is an increase in plasma Ac-globulin concentration.

The antibiotics penicillin, streptomycin, and aureomycin have been reported to produce hypercoagulable blood. The validity of this conclusion must be questioned, since there is no satisfactory knowledge about their action on blood coagulation.

When injected intravenously, protamine rapidly neutralizes the effect of injected heparin. The protamine itself disappears from the blood within a few hours. *In vitro,* protamine precipitates fibrinogen and profibrinolysin. In contrast to protamine the antiheparin effect of toluidine blue develops slowly but can still be detected 24 hours after an initial injection.

The bulk of evidence indicates that the cardiac glycosides produce hypercoagulable blood. This might be due to an increase in plasma Ac-globulin concentration. It has been stated that alpha tocopherol, by its action on plasma antithrombin, lowers the incidence of thrombosis. The validity of this claim rests largely upon clinical observations. Laboratory data do not supply an adequate theoretical basis to support the claim made for the effect of alpha tocopherol.

Heparin produces its anticoagulant action *in vivo* immediately. It has a twofold effect on the blood clotting mechanism. It blocks the activation of prothrombin and probably interferes with the action of thrombin on fibrinogen. Its duration of action, as measured by clotting effects, is limited to several hours. However, respository forms of heparin greatly prolong the action. The chief difficulty with synthetic heparin-like compounds has been their toxicity; however, a polysulfuric acid ester of polyanhydromanuronic acid has qualified for clinical trial.

There are certain nutritional factors which influence the coagulability of the blood, independently of vitamin K action. The sulfonamides, however, produce their effect by eliminating the intestinal bacteria which produce vitamin K.

From the available literature it is not possible to reach a conclusion concerning the effect of the adrenocorticotropic hormone. Probably hypercoagulability is one of its most important side effects. With the exception of chloroform, the anesthetics and respiratory gases do not produce profound effects. Deep chloroform anesthesia causes a decrease in the prothrombin, Ac-globulin and fibrinogen concentrations of the blood. This is the result of liver damage.

Under appropriate conditions epinephrine produces hypercoagulability of the blood. Acetylcholine, on the other hand, probably has the opposite effect and can apparently alter the blood so that fibrinolysis occurs in blood taken from the veins after acetylcholine-induced shock.

Some of the blood coagulation components have been prepared in concentrated form and their pharmacological effects have been studied in some detail, though not adequately as yet. Among these are thrombin, prothrombin, streptokinase-streptodornase, and thromboplastin.

REFERENCES

1. Addicks, K.: Versuche über die Blutgerinnung bei oral verbreichtem Euphyllin. Deutsches Arch. f. klin. Med., **140**: 117-121, 1922.

1a. Adamson, D. L., Weaver, R. T. and Jaimet, C. H.: A new view on the use of dicumarol in the pregnant patient. Canad. M. A. J., **61**: 6-10, 1949.

2. Aggeler, P. M. and Lucia, S. P.: Hemorrhagic disorders. The University of Chicago Press, 1949.

3. Alexander, B., Goldstein, R. and Landwehr, G.: The prothrombin conversion accelerator of serum (spca): Its partial purification and its properties compared with serum Ac-globulin. J. Clin. Investigation, **29**: 881–895, 1950.

4. Allen, E. V. and Barker, N. W.: A conjecture concerning benefits to man of artificially impaired coagulation of the blood. Proc. Staff Meet., Mayo Clin., **18**: 107–108, 1943.

5. Allen, E. V., Barker, N. W. and Waugh, J. M.: A preparation from spoiled sweet clover; (3,3′-methylene-bis-(4-hydroxycoumarin)) which prolongs coagulation and prothrombin time of the blood; a clinical study. J. Am. M. A., **120**: 1009–1015, 1942.

6. Allen, E. V., Hines, E. A., Jr., Kvale, W. F. and Barker, N. W.: The use of dicumarol as an anticoagulant: Experience in 2,307 cases. Ann. Int. Med., **27**: 371–381, 1947.

6a. Allen, J. G.: Personal communication.

6b. Allen, J. G.: Progress report. Abnormal bleeding. Argonne National Laboratory, Chicago, Ill., March, 1950.

7. Allen, J. G. and Jacobson, L. O.: Hyperheparinemia: Cause of the hemorrhagic syndrome associated with total body exposure to ionizing radiation. Science, **105**: 388, 1947.

8. Allen, J. G. and Livingstone, H.: Postoperative hypoprothrombinemia and anesthesia. Current Researches in Anesthesia & Analgesia, **20**: 298–299, 1941.

9. Allen, J. G., Bogardus, G., Jacobson, L. O. and Spurr, C. L.: Some observations on bleeding tendency in thrombocytopenic purpura. Ann. Int. Med., **27**: 382–395, 1947.

10. Allen, J. G., Grossman, B. J., Elghammer, R. M., Moulder, P. V., McKeen, C. L., Jacobson, L. O., Pierce, M., Smith, T. R. and Crosbie, J. M.: Abnormal bleeding: Response to treatment with toluidine blue and protamine sulfate. J. Am. M. A., **139**: 1251–1254, 1949.

11. Allen, J. G., Moulder, P. V., Elghammer, R. M., Grossman, B. J., McKeen, C. L., Sanderson, M., Egner, W. and Crosbie, J. A.: A protamine titration as an indication of a clotting defect in certain hemorrhagic states. J. Lab. & Clin. Med., **34**: 473–476, 1949.

12. Allen, J. G., Sanderson, M., Milham, M., Kirschon, A. and Jacobson, L. O.: Heparinemia (?) An anticoagulant in the blood of dogs with hemorrhagic tendency after total body exposure to roentgen rays. J. Exper. Med., **87**: 71–86, 1948.

13. Almquist, H. J.: Vitamin K. Physiol. Rev., **21**: 194–216, 1941.

14. Andreenko, G. V.: Influence of dicoumarin on prothrombin and thrombotropin concentration in the blood. Doklady Akad. Nauk. U. S. S. R., **61**: 1117–1120, 1948.

15. Ashworth, C. T. and McKemie, J. F.: Hemorrhagic complications with death probably from salicylate therapy: Report of 2 cases. J. Am. M. A., **126**: 806–810, 1944.

16. Askey, J. M. and Neurath, O.: Is digitalis indicated in myocardial infarction? J. Am. M. A., **14**: 1016–1018, 1945.

17. Astrup, T.: Heparin and the inhibition of blood clotting. Science, **90**: 36, 1939.

18. Astrup, T.: Biochemistry of blood coagulation. Acta physiol. Scandinav., Suppl. No. 21, 1944.

19. Astrup, T.: Blood clotting and related processes. Advances in Enzymology, **10**: 1–49, 1950.

20. Astrup, T. and Darling, S.: Antithrombin und Heparin. Naturwissenschaften, **29**: 300, 1941.

21. Astrup, T. and Darling, S.: Preparation and purificaton of thrombin. Acta physiol. Scandinav., **2**: 21–40, 1941.

22. Astrup, T. and Galsmar, I.: On the anticoagulant activity of heparin and synthetic polysaccharide sulfuric acids. Acta physiol. Scandinav., **8**: 361–364, 1944.

23. Astrup, T and Piper, J.: Cellulose sulfuric acids as anticoagulants. Acta physiol Scandinav., **9**: 351–356, 1945.

24. Astrup T. and Piper, J.: Interaction between fibrinogen and polysaccharide polysulfuric acids. Acta physiol. Scandinav., **11**: 211–220, 1946.

25. Astrup, T. and Volkert, M.: On the action of thrombin and thrombokinase in vivo. Acta med. Scandinav., **115**: 393–400, 1943.

26. Astrup, T., Galsmar, I. and Volkert, M.: Polysaccharide sulfuric acids as anticoagulants. Acta physiol. Scandinav., **8**: 215–226, 1944.

27. Axelrod, J., Cooper, J. R. and Brodie, B. B.: Estimation of dicumarol, 3,3′-methylenebis (4-hydroxycoumarin) in biological fluids. Proc. Soc. Exper. Biol. & Med., **70**: 693–695, 1949.

28. Axtrup, S.: Hypoprothrombinemia in penicillin treatment of acute infections in infants. Acta paediat., **35**: 351–354, 1948.

29. Aykut, R.: The effect of pentobarbital sodium on blood clotting time. Bull. faculte med. Istanbul, **11**: 121–128, 1948.

30. Bacq, Z. M.: La semicarbazone de l'adrenochrome. Compt. rend. Soc. de biol., **141**: 536–537, 1947.

31 Bacq, Z. M.: The metabolism of adrenaline. Pharmacol. Rev., **1**: 1–26, 1949.

32 Balkin, S. S. and Gootnick, A.: The effects of dicumarol on the electrocardiogram. J. Lab. & Clin. Med., **33**: 972–974, 1948.

33. Barclay, W. R.: The intravenous administration of p-aminosalicylic acid. Am. Rev. Tuberc., **60**: 385–386, 1949.

34. Barker, N. W., Allen, E. V. and Waugh, J. M.: The use of dicumarol (3,3′ methylenebis (4-hydroxycoumarin)) in the prevention of postoperative thrombophlebitis and pulmonary embolism. Proc. Staff Meet., Mayo Clin., **18**: 102–107, 1943.

35. Barker, N. W., Cromer, H. E., Hurn, M. and Waugh, J. M.: The use of dicumarol in the prevention of postoperative thrombosis and embolism with special reference to dosage and safe administration. Surgery, **17**· 207–217, 1945.

36. Barnes, A. C. and Erwin, H. K.: The effect of the anticoagulants on postpartum bleeding. Surg., Gynec. & Obst., **83**: 528–530, 1946.

37. Barsoe, O. C. and Selso, S.: On the anticoagulant action of synthetic polysaccharide polysulphuric acids Acta pharmacol. et toxicol., 2: 367–370, 1946.

38. Bartholomew, L. G. and Barker, N. W.: Personal communication.

39. Baserga, A. and de Nicola, P.: Le malattie emorragiche. Societa Editrice Libraria, 1950.

40. Battle, W. D., Capps, R. T., Orth, O. S. and Meyer, O. O.: The effects of 4-hydroxycoumarin anticoagulant No. 63* upon the prothrombin time in dogs and human beings. J. Lab. & Clin. Med., 35: 8–15, 1950.

41. Bauer, G., Boström, H., Jorpes, E. and Kallner, S.: Intramuscular administration of heparin. Acta med. Scandinav., 136: 188–198, 1950.

42. Baumann, C. A., Field, J. B., Overman, R. S. and Link, K. P.: Studies on the hemorrhagic sweet clover disease. X. Induced vitamin C excretion in the rat and its effect on the hypoprothrombinemia caused by 3,3′-methylenebis-(4-hydroxycoumarin). J. Biol. Chem., 146: 7–14, 1942.

43. Bayerle, H. and Marx, R.: Coumarin derivatives as antithrombin agents. Biochem. Ztschr., 319: 18–46, 1948.

44. Bayerle, H. and Marx, R.: Coumarin derivatives as antithrombins. II. Biochem. Ztschr., 319: 397–406, 1949.

45. Beattie, E. J., Cutler, E. C., Fauteux, M., Kinney, T. D. and Levine, H. D.: The use of dicumarol in experimental coronary occlusion. I. The ineffectiveness of dicumarol when ligation is the method of occlusion. Am. Heart J., 35: 94–105, 1948.

46. de Beer, E. J.: The use of Russell viper venom as a means of following the anticoagulant action of 3,3′-methylenebis (4-hydroxycoumarin) in the dog. J. Lab. & Clin. Med., 32: 90–93, 1947.

47. Bendix, R. M. and Necheles, H.: Wound healing and heparin, using heparin deposits. Surgery, 26: 799–805, 1949.

48. Bergström, S.: Naturwissenshaften, 23: 706, 1935 (Quoted by Jorpes (257)).

49. Bergström, S.: Über Polysaccharidesterschwefelsäuren mit Heparinwirkung. Ztschr. Physiol. Chem., 238: 163–168, 1936.

50. Bering, E. A., Jr.: Clinical uses of products made from human fibrinogen and thrombin. Bull. U. S. Army Med. Dept., No. 78: 53–56, 1944.

51. Best, C. H.: Heparin and thrombosis. Brit. M. J., 2: 977–981, 1938.

52. Best, C. H.: Heparin and thrombosis. Harvey Lect., Bull. New York Acad. Med., 17: 796–817, 1941.

53. Best, C. H. and Jaques, L. B.: Heparin in blood clotting and thrombosis. Ann. New York Acad. Sc., 49: 501–517, 1948.

54. Best, C. H., Cowan, C. and MacLean, D. L.: Heparin and the formation of white thrombi. J. Physiol., 92: 20–31, 1938.

55. Bickel, G. and Della Santa, R.: Un nouvel anticoagulant de synthese: l'ester ethylique de l'acide dioxycoumarinyl-acetique ou Tromexan. Revue Medicale de la Suisse Romande, 69: 546–560, 1949.

56. Biggs, R., Macfarlane, R. G. and Pilling, J.: Observations on fibrinolysis: Experimental activity produced by exercise or adrenaline. Lancet, 1: 402–405, 1947.

57. Black, S., Overman, R. S., Elvehjem, C. A. and Link, K. P.: The effect of sulfaguanidine on rat growth and plasma prothrombin. J. Biol. Chem., 145: 137–143, 1942.

58. Blaustein, A.: A new prothrombopenic agent. Canad. M. A. J., 62: 470–472, 1950.

59. Blaustein, A., Croce, J. J., Alberian, M. and Richey, N.: Preliminary report on the clinical use of a new anticoagulant, phenylindandione. Circulation, 1: 1195–1204, 1950.

60. Blood clotting and allied problems. Transactions of the First Conference, Josiah Macy Jr. Foundation, New York, 1948.

61. Blood clotting and allied problems. Transactions of the Second Conference, Josiah Macy Jr. Foundation, New York, 1949.

62. Blood clotting and allied problems. Transactions of the Third Conference, Josiah Macy Jr. Foundation, New York, 1950.

63. Blood clotting and allied problems. Transactions of the Fourth Conference, Josiah Macy Jr. Foundation, New York, 1951.

64. Blood, D. W. and Patterson, M. C.: Effect of aminophyllin on the coagulation of human blood. Proc. Soc. Exper. Biol. & Med., 69: 130–133, 1948.

65. Blumgart, H. L., Freedberg, A. S., Zoll, P. M., Lewis, H. D. and Wessler, S.: Effect of Dicumarol on the heart in experimental acute coronary occlusion. Tr. A. Am. Physicians, 60: 227–231, 1947.

66. Bollman, J. L.: Failure of Dicumarol and heparin to modify experimental renal infarction. Proc. Staff Meet., Mayo Clin., 19: 248–250, 1944.

67. Bollman, J. L. and Preston, F. W.: The effects of experimental administration of dicoumarin. J. Am. M. A., 120: 1021–1025, 1942.

68. Borgström, S.: Prothrombin index after operation. Acta chir. Scandinav., 89: 68–74, 1943.

69. Bose, A. N.: Ann. Biochem. Exper. Med., (India) 5: 105, 1945. (Quoted by Munro and Lupton (370)).

70. Boyd, E. J. and Warner, E. D.: Effect of vitamin K on Dicumarol-induced hypoprothrombinemia in rats. J. Lab. & Clin. Med., 33: 1431–1437, 1948.

71. de M. Braganca, B. and Radhakrishna Rao, M. V.: Hypoprothrombinemia produced by sulfathiazole in rats on a diet free of vitamin K and cured by synthetic vitamin K. Indian J. M. Research, 35: 15–21, 1947.

72. Brambel, C. E.: Thromboplastin reagent. Development of a more suitable preparation for measuring accelerated clotting tendency and for use following administration of dicoumarin (3,3′-methylenebis 4-hydroxycoumarin). Arch. Surg., 50: 137–147, 1945.

73. Brambel, C. E. and Hunter, R. E.: Effect of Dicumarol on the nursing infant. Am. J. Obst. & Gynec., 59: 1153–1159, 1950.

74. Brambel, C. E. and Loker, F. F.: Significance of variations of prothrombin activity of dilute plasma. Proc. Soc. Exper. Biol. & Med., **53:** 218–220, 1943.

75. Brambel, C. E. and Loker, F. F.: Application of dicoumarin (3,3′-methylenebis (4-hydroxycoumarin)) in trauma and gangrene. Arch. Surg., **48:** 1–16, 1944.

76. Brambel, C. E., Hunter, R. E. and Fitzpatrick, P.: Prophylactic use of anticoagulants in the puerperal period (Dicumarol, heparin and Link compound 63). Bull. School Med. Univ. Maryland, **35:** 91–103, 1950.

77. Brettspraak, R. W. and Greenspan, F. R.: The effect of aminophylline on the prothrombin time in man. Am. J. M. Sc., **212:** 476–478, 1946.

78. Brinkhous, K. M.: Plasma prothrombin: vitamin K. Medicine, **19:** 329–416, 1940.

79. Brinkhous, K. M.: Clotting defect in hemophilia: deficiency in a plasma factor required for platelet utilization. Proc. Soc. Exper. Biol. & Med., **66:** 117–120, 1947.

80. Brinkhous, K. M., Smith, H. P. and Warner, E. D.: Plasma prothrombin level in normal infancy and in hemorrhagic disease of the newborn. Am. J. M. Sc., **193:** 475–480, 1937.

81. Brinkhous, K. M., Smith, H. P., Jr., Warner, E. D. and Seegers, W. H.: Heparin and blood clotting. Science, **90:** 539, 1939.

82. Brinkhous, K. M., Smith, H. P., Warner, E. D. and Seegers, W. H.: The inhibition of blood clotting: An unidentified substance which acts in conjunction with heparin to prevent the conversion of prothrombin into thrombin. Am. J. Physiol., **125:** 683–687, 1939.

83. Burke, G. E. and Wright, I. S.: A new coumarin anticoagulant (Tromexan) 4,4′-dihydroxydicumarnyl ethyl acetate: Preliminary report of its action. Bull. New York Acad. Med., **26:** 264–265, 1950.

84. Burke, G. E. and Wright, I. S.: Tromexan (3,3′-carboxymethylenebis-(4′-dihydroxycoumarin) ethyl ester acetic acid. Experimental and clinical properties. Circulation, **3:** 164–170, 1951.

85. Burstein, M.: La coagulation du sang. Masson and Cie, 1949.

86. Burt, C. C., Wright, H. P. and Kubik, M.: Clinical tests of a new coumarin substance. A report to the Medical Research Council. Brit. M. J., **2:** 1250–1260, 1949.

87. Butsch, W. L. and Stewart, J. D.: Clinical experiences with dicumarin, 3,3′-methylenebis-(4-hydroxycoumarin). J. Am. M. A., **120:** 1025–1028, 1942.

88. Butt, H. R. and Snell, A. M.: Vitamin K. W. B. Saunders Co., Philadelphia, 1941.

89. Butt, H. R., Leake, W. H., Solley, R. F., Griffith, G. C., Huntington, R. W. and Montgomery, H.: Studies in rheumatic fever. I. The physiologic effect of sodium salicylate on the human being, with particular reference to the prothrombin level of the blood and the effect on hepatic parenchyma. J. Am. M. A., **128:** 1195–1200, 1945.

90. Byron, R. L., Jr., McGee, J. and Bell, H. G.: Thrombin in the control of upper gastrointestinal hemorrhage. Report of three cases. California Med., **71:** 419–420, 1949.

91. Cahan, A.: Hemorrhage and purpura caused by dicoumarin. Report of a case. New England J. Med., **228:** 820–822, 1943.

92. Canessa, I.: Vitamin K. Pubs. med. exptl., Univ. Chile, **2:** 9–312, 1947.

93. Cannon, W. B. and Gray, H.: Factors affecting the coagulation time of blood. II. The hastening or retarding of coagulation by adrenalin injections. Am. J. Physiol., **34:** 232–242, 1914.

94. Cannon, W. B. and Mendenhall, W. L.: Factors affecting the coagulation time of blood. I. The graphic method of recording coagulation used in these experiments. Am. J. Physiol., **34:** 225–231, 1914.

95. Cannon, W. B. and Mendenhall, W. L.: Factors affecting the coagulation time of blood. III. The hastening of coagulation by stimulating the splanchnic nerves. Am. J. Physiol., **34:** 243–250, 1914.

96. Cannon, W. B. and Mendenhall, W. L.: Factors affecting the coagulation time of blood. IV. The hastening of coagulation in pain and emotional excitement. Am. J. Physiol., **34:** 251–261, 1914.

97. Canova, F.: Streptomycin, blood calcium and blood coagulation. Boll. soc. ital. biol. sper., **24:** 976–978, 1948.

98. Carter, J. C., Bingham, J. B. and Meyer, O. O.: Studies on the hemorrhagic agent 3,3′-methylenebis (4-hydroxycoumarin). IV. The pathologic findings after the administration of Dicumarol. Am. J. Clin. Path., **20:** 651–657, 1944.

99. Cathcart, R. T. and Blood, D. W.: Effect of digitalis on the clotting of the blood in normal subjects and in patients with congestive heart failure. Circulation, **1:** 1176–1181, 1950.

100. Chargaff, E.: The protamine salts of phosphatides, with remarks on the problem of lipoproteins. J. Biol. Chem., **125:** 661–670, 1938.

101. Chargaff, E.: The coagulation of blood. Chapter in Advances in Enzymology, Interscience Publishers, Inc., 1945.

102. Chargaff, E. and Olson, K. B.: Studies on the chemistry of blood coagulation. VI. Studies on the action of heparin and other anticoagulants. The influence of protamine on the anticoagulant effect in vivo. J. Biol. Chem., **122:** 153–167, 1937.

103. Chargaff, E., Moore, D. H. and Bendich, A.: Ultracentrifugal isolation from lung tissue of macromolecular protein component with thromboplastic properties. J. Biol. Chem., **145:** 593–603, 1942.

104. Chargaff, E., Ziff, M. and Moore, D. H.: Studies on the chemistry of blood coagulation. XII. An electrophoretic study of the effect of anticoagulants on human plasma proteins, with remarks on the separation of the heparin complement. J. Biol. Chem., **139:** 383–405, 1941.

105. Christensen, L. R.: Methods for measuring the activity of components of the streptococcal fibrinolytic system, and streptococcal desoxyribonuclease. J. Clin. Investigation, **28:** 163–172, 1949.

106. Clark, A. M., Milne, G. R. and Todd, J. P.: Fixation of skin-grafts with human plasma and thrombin. Lancet, **1:** 498–502, 1945.

107. Clark, B. B. and Spitalny, M.: Prothrombinopenic activity of the salicylates and pharmacologically related drugs. Federation Proc., 5: 171–172, 1946.

108. Clark, W. G.: Effect of rutin on dicoumarolism. Proc. Soc. Exper. Biol. & Med., 69: 97–98, 1948.

109. Clausen, F. W. and Jager, B. V.: The relation of the plasma salicylate level to the degree of hypoprothrombinemia. J. Lab. & Clin. Med. 31: 428–436, 1946.

110. Clay, H. L. and Dickinson, L. D.: Needle biopsy of the liver using oxidized cellulose and thrombin to prevent hemorrhage. J. Lab. & Clin. Med., 34: 422–424, 1949.

111. Coburn, A. F.: Salicylate therapy in rheumatic fever. Bull. Johns Hopkins Hosp., 73: 435–464, 1943.

112. Cohn, E. J.: The properties and functions of the plasma proteins, with a consideration of the methods for their separation and purification. Chem. Rev., 28: 395–417, 1941.

113. Cohn, E. J.: Blood proteins and their therapeutic value. Science, 101: 51–56, 1945.

114. Cohn, E. J.: The separation of blood into fractions of therapeutic value. Ann. Int. Med., 26: 341–352, 1947.

115. Coleman, F. C.: The prothrombin time in Dicoumarol therapy. Ann. Int. Med., 30: 895–902, 1949.

116. Coombs, A. J.: Delayed bleeding following removal of tonsils and adenoids. Laryngoscope, 60: 296–306, 1950.

117. Coombs, F. S., Jr., Higley, C. S. and Warren, H. A.: The magnitude of salicylate hypoprothrombinemia. Proc. Central Soc. Clin. Research 18: 68–69, 1945.

118. Copley, A. L.: Studies on human placental thromboplastin in vitro and in vivo. Science, 101: 436–437, 1945.

119. Copley, A. L.: On a new blood coagulation phase and the effect of Dicumarol and other hemorrhagic agents in rabbits. Proc. XVIII International Physiological Congress: 165–166, 1950.

120. Copley, A. L. and Robb, T. P.: Studies on platelets. II. The effect of heparin on the platelet count in vitro. Am. J. Clin. Path., 12: 416–423, 1942.

121. Copley, A. L. and Robb, T. P.: Studies on platelets. III. The effect of heparin in vivo on the platelet count in mice and dogs. Am. J. Clin. Path., 12: 563–570, 1942.

122. Copley, A. L. and Schnedorf, J. G.: The rate of excretion of heparin in the urine following its intravenous injection in the anesthetised dog. Am. J. Physiol., 133: 562–565, 1941.

123. Correll, J. T. and Wise, E. C.: The stability of thrombin, penicillin, or streptomycin, in the presence of gelatin sponge, oxidised cellulose or each other. Surg., Gynec. & Obst., 85: 211–213, 1947.

124. Cosgriff, S. W., Diefenbach, A. F. and Vogt, W., Jr.: Hypercoagulability of the blood associated with ACTH and cortisone therapy. Am. J. Med., 9: 752–756, 1950.

125. Cowley, L. L. and Lam, C. R.: The neutralization of heparin by protamine. Surgery, 24: 97–99, 1948.

126. Cromer, H. E., Jr. and Barker, N. W.: Effect of large doses of menadione bisulfite (synthetic vitamin K) on excessive hypoprothrombinemia induced by Dicumarol. Proc. Staff Meet., Mayo Clin., 19: 217–223, 1944.

127. Cronkite, E. P., Deaver, J. M. and Lozner, E. L.: Experiences with use of thrombin with and without soluble cellulose for local hemostasis. War Med., 5: 80–82, 1944.

128. Cronkite, E. P., Lozner, E. L. and Deaver, J. M.: Use of thrombin and fibrinogen in skin grafting. J. Am. M. A., 124: 976–978, 1944.

129. Cullen, S. C., Ziffren, S. E., Gibson, R. B. and Smith, H. P.: Anesthesia and liver injury: With special reference to plasma prothrombin levels. J. Am. M. A., 115: 991–993, 1940.

130. Dale, D. U. and Jaques, L. B.: The prevention of experimental thrombosis by dicoumarin. Canad. M. A. J., 46: 546–548, 1942.

131. Daly, B. M., Johnston, C. G. and Penberthy, G. C.: The management of patients with bleeding from the upper gastrointestinal tract with buffer and thrombin solution. Ann. Surg., 129: 832–839, 1949.

132. Dam, H.: The discovery of vitamin K. Nobel Lecture, December 12, 1946.

133. Dam, H.: Vitamin K. Vitamins & Hormones, 6: 28–53, 1948.

134. Dam, H., Larsen, H. and Plum, P.: Om fibrinopeni ved for tidlig løsning of placenta. Saertryk af Ugeskrift for Laeger, 103: 257–264, 1941.

135. Davidoff, L. M., Seegal, B. C. and Seegal, D.: The Arthus phenomenon. Local anaphylactic inflammation in the rabbit brain. J. Exper. Med., 55: 163–168, 1932.

136. Davidson, C. S. and MacDonald, H.: Effect of vitamin K_1 oxide on hypoprothrombinemia induced by Dicumarol. New England J. Med., 229: 353–355, 1943.

137. Davis, A. and Porter, M.: Dicoumarin in the treatment of puerperal thrombosis. Brit. M. J., 1: 718–719, 1944.

138. Dell'Aquila, M. D. and Lojacono, I.: Action of sodium salicylate on blood prothrombin titer. Bull. soc. ital. biol. sper., 22: 295–296, 1946.

139. Della Santa, R.: Un nouvel anticoagulant de synthese; le tromexan (G 11705). Schweiz. med. Wchnschr., 79: 195–206, 1949.

140. Della Santa, R. and von Kaulla, K. N.: L'hypoprothrombinemie provoquie chez les hepatiques. Helv. med. Acta, 16: 251–257, 1949.

141. Derouaux, G.: The hemostatic action of derivatives of adrenochrome. Arch. internat. de pharmacodyn. et de thérap., 69: 142, 1943.

142. Deutsch, E.: Erfahrungen mit Tromexan, einem neuen Anticoagulans der Dicumarolreihe. Schweiz. med. Wchnschr., 79: 1010–1025, 1949.

143. Deutsch, E.: Die Hemmkörper Hämophilie, Springer-Verlag, 1950.

144. Deutsch, E. and Huebef, E. F.: Ueber die Behandlung der Coronarthrombose und des Herzmuskelinfarktes mit gerinnungshemmenden Substanzen. Wien. klin. Wchnschr., 62: 277–281, 1950.

145. Dieckman, W. F.: The toxemias of pregnancy, C. V. Mosby, 1941.

146. Dienst, A.: Experimentelle Studien über die ätiologische Bedeutung des Fibrinferments und Fibrinogens für die Eklampsie. Arch. f. Gynak., 96: 43–170, 1912.

147. Doisy, E., Binkley, S. B. and Thayer, S. A.: Vitamin K. Chem. Rev., 28: 477–517, 1941.

148. Dolkart, R. E., Halpern, D., Larkin, M., Dey, F. L. and de Takats, G.: Effect of penicillin on the clotting activity of blood in normal human subjects. J. Pharmacol. & Exper. Therap., 96: 291–294, 1949.

149. Donatelli, L. and Pasquinucci.: Streptomycin and blood coagulation. Boll. Soc. ital. biol. sper., 24: 963–964, 1948.

150. Draper, A. J.: Dicumarol poisoning. J. Am. M. A., 136: 171–172, 1948.

151. Duff, I. J. and Shull, W. H.: Fatal hemorrhage in Dicumarol poisoning. With respect to necropsy. J. Am. M. A., 139: 762–766, 1949.

152. Editorial: Antibiotics and blood coagulation. J. Am. M. A., 141: 924, 1949.

153. Editorial: Biological competition between structurally related compounds: Clinical implications. Ann. Int. Med., 30: 867–870, 1949.

154. Editorial: Heparinemia. Blood, 3: 1304–1306, 1948.

155. Ellis, M. M. and Barlow, O. W.: Barbital narcosis. II. Blood sugar and blood coagulation time during barbital hypothermia. J. Pharmacol. & Exper. Therap., 24: 259–264, 1924.

156. Evans, J. A. and Boller, R. J.: Subcutaneous use of heparin in anticoagulation therapy. J. Am. M. A., 131: 879–882, 1946.

157. Fahey, J. L., Olwin, J. H. and Ware, A. G.: Effect of Dicumarol on Ac-globulin and prothrombin activity. Proc. Soc. Exper. Biol. & Med., 69: 491–494, 1948.

158. Falk, O. P. J.: Treatment of coronary artery disease; Dicumarol therapy. J. Am. M. A., 134: 491–495, 1947.

159. Fallon, J. and Croskery, W. F.: Plasma fractions—"fibrin foam," thrombin, and "fraction no. 1"—in general surgery. Surg., Gynec. & Obst., 84: 361–365, 1947.

160. Fantl, P.: Inhibition of blood coagulation by coumarin compounds. Australian J. Sc., 6: 23–25, 1943.

161. Fantl, P. and Nance, M. H.: The hypoprothrombinaemic effect of 3,3′-ethylidene-bis-4-hydroxycoumarin (E.D.C.): An experimental and clinical study. M. J. Australia, 2: 133–136, 1947.

162. Fantl, P. and Nance, B. A.: The action of protamine on blood plasma. Australian J. Exper. Biol. & M. Sc., 28: 253–259, 1950.

163. Fantl, P., Everard, B. A. and Nelson, J. F.: The question of arginine linkage in salmine. Australian J. Sc., 12: 145–146, 1949.

164. Fashena, G. T. and Walker, J. N.: Salicylate intoxication. Am. J. Dis. Child., 68: 369–375, 1944.

165. Favre-Gilly, J.: Les etats hemorragiques et la notion de fibrinopenie, Vigot Freres, 1947.

166. Ferguson, J. H.: The blood calcium and the calcium factor in blood coagulation. Physiol. Rev., 16: 640–670, 1936.

167. Ferguson, J. H.: Blood: Coagulation, biophysical characters, and formed elements. Ann. Rev. Physiol., 2: 71–108, 1940.

168. Ferguson, J. H.: The action of heparin, serum albumin (crystalline), and salmine on blood-clotting mechanisms (in vitro). Am. J. Physiol., 130: 759–770, 1940.

169. Ferguson, J. H.: Blood coagulation thrombosis, and hemorrhagic disorders. Ann. Rev. Physiol., 8: 231–262, 1946.

170. Ferry, J. D. and Shulman, S.: The conversion of fibrinogen to fibrin. I. Influence of hydroxy compounds on clotting time and clot opacity. J. Am. Chem. Soc., 71: 3198–3204, 1949.

171. Fidlar, E. and Jaques, L. B.: The effect of commercial heparin on the platelet count. J. Lab. & Clin. Med., 33: 1410–1423, 1948.

172. Field J. B.: Hypoprothrombinemia induced in suckling rats by feeding 3,3′-methylenebis-(4-hydroxycoumarin) and acetylsalicylic acid to their mothers. Am. J. Physiol., 143: 238–242, 1945

173. Field, J. B. and Link, K. P.: Note on hyperprothrombinemia induced by vitamin K. J. Biol. Chem., 156: 739–741, 1944.

174. Field, J. B., Larsen, E. G., Spero, L. and Link, K. P.: Studies in the hemorrhagic sweet clover disease. XIV. Hyperprothrombinemia induced by methylxanthines and its effect on the action of Dicumarol. J. Biol. Chem., 156: 725–737, 1944.

175. Field, J. B., Overman, R. S. and Baumann, C. A.: Prothrombin activity during pregnancy and lactation. Am. J. Physiol., 137: 509–514, 1942.

176. Field, J. B., Spero, L. and Link, K. P.: Hypoprothrombinemia induced in the dog by acetylsalicylic acid. Am. J. Physiol., 159: 40–46, 1949.

177. Field, J. B., Sveinbjornsson, A. and Link, K. P.: Increased plasma fibrinogen induced by methylxanthines. J. Biol. Chem., 159: 525–528, 1945.

178. Fischer, A.: Die Bindung von Heparin an Eiweiss. Biochem. Ztschr., 278: 133–160, 1935.

179. Fischer, P. and deLandtsheer, L.: Metabolism of adrenochrome and of trihydroxymethyl indole in the rabbit. Experientia, 6: 305, 1950.

180. Fischer, P. and Lecomte, J.: Metabolisme de la semicarbazone de l'adrenochrome absorbie per os par l'homme normal. Compt. rend. Soc. de biol., 142: 1446–1448, 1948.

181. Fleming, A. and Lish, E. W.: Influence of penicillin on the coagulation of blood. Brit. M. J., 2: 242–243, 1947.

182. Flinn, J. H.: The effect of digitalis on the coagulability of blood. Collected Papers, Mayo Clinic and Foundation, 40: 380–381, 1949.

183. Foley, W. T and Wright, I. S. The treatment of cerebral thrombosis and embolism with anticoagulant drugs: preliminary observations. M. Clin. North America, 34: 909–924, 1950.

184. Fox, S. L.: The use of thrombin in rhinologic surgery—A preliminary report. Laryngoscope, 56: 48–53, 1946.

185. Fox, S. L.: The use of thrombin in nasal surgery. Ann. Otol., Rhin. & Laryng., 56: 127–133, 1947.

186. Frantz, V. K.: Hemostasis. Ann. Rev. Physiol., 10: 445–452, 1948.
187. Friedrich, L. and Policzer, M.: Uses of thrombin in liver puncture. Lancet, 1: 523–524, 1949.
188. Fulton, L. D. and Page, E. W.: Nature of refractory state following sublethal dose of human placental thromboplastin. Proc. Soc. Exper. Biol. & Med., 68: 594–596, 1948.
189. Galt, J. and Hunter, R. B.: The effect of aureomycin on certain liver function tests and blood coagulation. Am. J. M. Sc., 220: 508–510, 1950.
190. Gerber, C. F. and Blanchard, E. W.: The effect of certain substances on clotting time, in vitro. Am. J. Physiol., 144: 447–456, 1945.
191. Gerendas, M. and Csapo, A.: Intravenöse Thrombinwirkung. Arch. Biol. Hungarica, 18: 181–185, 1948.
192. Gianella, L.: Klinische Untersuchungen mit Anticoagulantien. Gynaecologia, 128: 62, 1949.
193. Gianella, C. V. and von Kaulla, K. N.: Über die Nachweisbarkeit des Äthylesters der 3,3′-Dicumarinylessigsäuer und des Dicumarols im menschlichen Blut. Experientia, 5: 125–126, 1949.
194. Gibson, P. C.: Aspirin in the treatment of vascular diseases. Lancet, 2: 1172–1174, 1949.
195. Gilbert, N. C. and Nalefski, L.: The effect of the anticoagulant drugs upon the coronary flow. J. Lab. & Clin. Med., 33: 1455–1456, 1948.
196. Gilbert, N. C., Dey, F. and Trump, R.: The effect of the methylated xanthines on the clotting time of the blood. J. Lab. & Clin. Med., 32: 28–33, 1947.
197. Glavind, J.: Studies on the coagulation of crustacean blood, Nyt Nordisk Forlag, 1948.
198. Goodwin, J. F. and Macgregor, A. G.: Anticoagulant therapy with heparin in Pitkin's menstruum. Lancet, 2: 667–671, 1950.
199. Govan, C. D.: The effect of salicylate administration on the prothrombin time. J. Pediat., 29: 629–636, 1946.
200. Grabfield, G. P.: Factors affecting the coagulation time of blood. IX. The effect of adrenin on the factors of coagulation. Am. J. Physiol., 42: 46–55, 1917.
201. Gray, H. and Lunt, L. K.: Factors affecting the coagulation time of blood. V. The effects of hemorrhage before and after exclusion of abdominal circulation, adrenals, or intestine. Am. J. Physiol., 34: 332–351, 1914.
202. Green, H. J. and Loewe, L.: Anticoagulant therapy with heparin/Pitkin menstruum in the thromboembolic disease complicating the puerperium and gynecologic surgery. Am. J. Obst. & Gynec., 54: 958–969, 1947.
203. Guest, M. M.: Personal communication and unpublished data.
204. Guest, M. M., Ware, A. G. and Seegers, W. H.: A quantitative study of antifibrinolysin in chick plasma: Increase in antifibrinolysin activity during pteroylglutamic acid deficiency. Am. J. Physiol., 150: 661–669, 1947.
205. Halse, T.: Dicumarol properties and application. Med. Klin., 43: 177–185, 1948.
206. Ham, T. H. and Curtis, F. C.: Plasma fibrinogen response in man. Influence of the nutritional state, induced hyperpyrexia, infectious disease and liver damage. Medicine, 17: 413–446, 1938.
207. Hammarsten, J. F.: Hemopericardium without rupture of the heart following Dicumarol therapy for myocardial infarction. Minnesota Med., 32: 1003–1004, 1949.
208. Hanzlik, P. J.: Action and uses of salicylates and cincophen in medicine, Williams and Wilkins Co., Baltimore, Maryland, 1927.
209. Hanzlik, P. J. and Karsner, H. T.: Anaphylactoid phenomena from various agents injected intravenously. J. Pharmacol. & Exper. Therap., 23: 173–235, 1924.
210. Hanzlik, P. J. and Karsner, H. T.: Effects from intraperitoneal injection of various agents causing anaphylactoid phenomena. J. Pharmacol. & Exper. Therap., 23: 243–260, 1924.
211. Harned, B. K., Cunningham, R. W., Clark, M. C., Cosgrove, R., Hine, C. H., McCauley, W. J., Stokey, E., Vessey, R. E., Yuda, N. N. and Subba-Row, Y.: The pharmacology of duomycin (aureomycin). Ann. New York Acad. Sc., 51: 182–210, 1948.
212. Harvey, W. P. and Finch, C. A.: Dicumarol prophylaxis of thromboembolic disease in congestive heart failure. New England J. Med., 242: 208–211, 1950.
213. Hendrickson, F. C.: Topical use of clotting agents in surgery of the prostate gland. J. Urol., 55: 613–617, 1946.
214. Herrell, W. E.: Observations on the clinical use of aureomycin. Proc. Staff Meet., Mayo Clin., 24: 612–618, 1949.
215. Higly, C. S.: Use of salicylates in rheumatic fever. J. Am. M. A., 129: 1123–1124, 1945.
216. Hill, J. M.: Personal communication, December, 1950.
217. Hilton, J. H. B., Cameron, W. M., Townsend, S. R. and Mills, E. S.: Dicoumarol in acute coronary occlusion. Canad. M. A. J., 60: 134–135, 1949.
218. Hilton, J. H. B., Cameron, W. M., Townsend, S. R. and Mills, E. S.: Problems involved in the use of Dicoumarol in acute coronary thrombosis. Canad. M. A. J., 60: 386–388, 1949.
219. Hines, E. A., Jr. and Barker, N. W.: Anticoagulant therapy in chronic cardiovascular diseases. M. Clin. North America, 33: 335–345, 1949.
220. Hines, L. E. and Kessler, D. L.: The effect of penicillin on heparin tolerance. J. Am. M. A., 128: 794–796, 1945.
221. Hoefner, P. F. A., Putnam, T. J. and Gray, M. G.: Experimental "encephalitis" produced by intravenous injection of various coagulants. Arch. Neurol. & Psychiat., 39: 799–812, 1938.
222. Hogben, C. A. M. and Allen, E. V.: The relationship between prothrombin time and bleeding in the clinical use of Dicumarol after operation. Circulation, 2: 369–372, 1950.
223. Holden, W. D.: Treatment of deep venous thrombosis with reference to subcutaneous injection of heparin and use of Dicumarol. Arch. Surg., 54: 183–187, 1947.
224. Holland, H. and Gross, E. G.: The effect of the xanthine derivatives on the prothrombin and plasma clotting times in dogs and in human subjects. J. Iowa M. Soc., 38: 183–187, 1948.

225. Holoubek, J. E., Hendrick, J. V. and Hollis, W. J.: Toluidine blue in bleeding associated with thrombopenia. J. Am. M. A., **139:** 214–216, 1949

226. Honorato, R.: The plasmatic cofactor of thromboplastin: Its adsorption, with prothrombin and fibrinogen, by alumina and tricalcium phosphate gels. Am. J. Physiol., **150:** 381–388, 1947.

227. Honorato, R.: Changes in the prothrombin time induced by methylxanthines. Role of the plasma cofactor of thromboplastin. Arch. Biochem., **22:** 345–352, 1949.

228. Honorato, R. and Gomez, C.: Thrombosis experimental y su relation con los digitalicos. Rev. Soc. argent. de biol., **24:** 295–300, 1948.

229. Honorato, R. and Lopetegui, M.: Relation of digitalis substances to blood coagulation, and their antagonism toward Dicumarol. Rev. Soc. argent. de biol., **24:** 286–294, 1948.

230. Horn, Z., Gerendas, M. and Borsodi, L.: The action of toluidine blue and thrombokinase on the course of thrombin inactivation. Experientia, **4:** 402, 1948.

231. Howell, W. H.: Theories of blood coagulation. Physiol. Rev., **15:** 435–470, 1935.

232. Huebner, C. F. and Link, K. P.: Studies on the hemorrhagic sweet clover disease. IV. Synthesis of the δ-Diketone derived from the hemorrhagic agent through alkaline degradation. J. Biol. Chem., **138:** 529–534, 1941.

233. Huebner, C. F., Sullivan, W. R., Stahmann, M. A. and Link, K. P.: Studies on 4-hydroxycoumarin. III. Dehydration of the aldehyde condensation products. J. Am. Chem. Soc., **65:** 2292-2296, 1943.

234. Hurn, M., Barker, N. W. and Magath, T. B.: Prothrombin-time determinations following the administration of Dicoumarol, with special reference to the variability of thromboplastin. Proc. Staff Meet., Mayo Clin., **19:** 507–512, 1944.

235. Hurn, M., Barker, N. W. and Magath, T. B.: The determination of prothrombin time following the administration of Dicoumarol, 3,3′-methylenebis (4-hydroxycoumarin), with special reference to thromboplastin. J. Lab. & Clin. Med., **30:** 432–437, 1945.

236. Ikawa, M., Stahmann, M. A. and Link, K. P.: Studies on 4-hydroxycoumarins. V. The condensation of α, β unsaturated ketones with 4-hydroxycoumarin. J. Am. Chem. Soc., **66:** 902–906, 1944.

237. Influence of digitalis on blood clotting. J. Am. M. A., **131:** 353, 1946.

238. Ingraham, F. D. and Bailey, O. T.: The use of products prepared from human fibrinogen and human thrombin in neurosurgery. Fibrin foams as hemostatic agents; fibrin films in repair of dural defects and in prevention of meningocerebral adhesions. J. Neurosurg., **1:** 23–39, 1944.

239. Irish, U. D. and Jaques, L. B.: The effect of Dicumarol upon plasma fibrinogen. Am. J. Physiol., **143:** 101–104, 1945.

240. Jacox, R. F.: Studies on the activation of a serum "prothrombin-conversion factor". J. Clin. Investigation, **28:** 492–504, 1949.

241. James, D. F., Bennett, I. L., Jr., Scheinberg, P. and Butler, J. J.: Clinical studies on Dicumarol hypoprothrombinemia and vitamin K preparations. I. Superiority of vitamin K_1 oxide over menadione sodium bisulfite U.S.P. and synkavite in reversing Dicumarol hypoprothrombinemia. Arch. Int. Med., **83:** 632–652, 1949.

242. Jansen, K. F.: Dikumarin, (3,3′-metylen-bis-(4-oxykumarin)) dets anverdelse i thrombosebehandlingen. Nord. Med., **20:** 1993–1996, 1943.

243. Jansen, K. F. and Jensen, K. A.: Constitution and hemorrhagic action of 3,3′-methylenebis (4-hydroxycoumarin). Ztschr. physiol. Chem., **277:** 66–73, 1942.

244. Jaques, L. B.: The effect of intravenous injections of heparin in the dog. Am. J. Physiol., **125:** 98–107, 1939.

245. Jaques, L. B.: The reaction of heparin with proteins and complex bases. Biochem. J., **37:** 189–195, 1943.

246. Jaques, L. B.: A study of the toxicity of the protamine salmine. Brit. J. Pharmacol., **4:** 135–144, 1949.

247. Jaques, L. B. and Dunlop, A. P.: The effect of calcium concentration on the prothrombin time of dogs treated with Dicumarol. Am. J. Physiol., **143:** 355–360, 1945.

248. Jaques, L. B. and Lepp, E.: Action of sodium salicylate on prothrombin time in rabbits. Proc. Soc. Exper. Biol. & Med., **66:** 178–181, 1947.

249. Jaques, L. B. and Ricker, A. G.: The relationship between heparin dosage and clotting time. Blood, **3:** 1197–1212, 1948.

250. Jaques L. B., Bruce-Witford, M. and Ricker, A. G.: The metachromatic activity of heparin. Rev. canad. de biol., **6:** 740–754, 1947.

251. Jaques, L. B., Charles, A. F. and Best, C. H.: The administration of heparin. Acta med. Scandinav., Suppl. **90:** 190–207, 1938.

252. Jaques, L. B., Fidlar, E., Feldsted, E. T. and Macdonald, A. G.: Silicones and blood coagulation. Canad. M. A. J., **55:** 26–31, 1946.

253. Jaques, L. B., Gordon, E. and Lepp, E.: A new prothrombopenic drug, phenylindanedione. Canad. M. A. J., **62:** 465–470, 1950.

254. Jaques, L. B., Taylor, E. and Lepp, E.: Action of phenylindanedione on prothrombin time. Federation Proc., **8:** 81, 1949.

255. Javert, C. T. and Macri, C.: Prothrombin concentration in normal pregnancy. Am. J. Obst. & Gynec., **42:** 415–419, 1941.

256. Jensen, R., Snellman, O. and Sylvén, B.: On the inhomogeneity of commercial heparin preparations from the physicochemical point of view. J. Biol. Chem., **174:** 265–271, 1948.

257. Jorpes, J. E.: Heparin in the treatment of thrombosis, Oxford University Press, 1946.

258. Jorpes, J. E.: The origin and the physiology of heparin: The specific therapy in thrombosis. Ann. Int. Med., **27:** 361–370, 1947.

259. Jorpes, J. E.: On the dosage of the anticoagulants, heparin and Dicumarol, in the treatment of thrombosis. Acta chir. Scandinav., Suppl. **149**: 1950.

260. Jorpes, J. E. and Gardell, S.: On heparin monosulfuric acid. J. Biol. Chem., **176**: 267–276, 1948.

261. Jorpes, E., Edman, P. and Thaning, T.: Neutralisation of action of heparin by protamine. Lancet, **2**: 975–976, 1939.

262. Jorpes, E., Kallner, S. and Boström, H.: Protamine for intravenous use in man and in animal experiments. Acta med. Scandinav., **84**: 1–4, 1949.

263. Jubeliber, R. A. and Glueck, H. I.: Capillary fragility studies (Göthlin test) on one hundred patients receiving Dicumarol. J. Lab. & Clin. Med., **34**: 448–457, 1949.

264. Julen, C., Snellman, O. and Sylvén, B.: Cytological and fractionation studies on the cytoplasmic constituents of tissue mast cells. Acta physiol. Scandinav., **19**: 289–305, 1950.

265. Jürgens, R. and Studer, A.: Zur Wirkung des Thrombins. Helv. Physiol. et Pharm. Acta, **6**: 130–149, 1948

266. Kabat, H., Stohlman, E. F and Smith, M. I.: Hypoprothrombinemia induced by administration of indandione derivatives. J. Pharmacol. & Exper. Therap., **80**: 160–170, 1944.

267. von Kaulla, K. N.: Betrachtungen zur postnarkotischen Fibrinolyse. Schweiz. med. Wchnschr., **77**: 313–325, 1947.

268. von Kaulla, K. N.: Klinische Fortschritte auf dem Gebiete der Blutgerinnung, Urban and Schwarzenberg, 1949.

269. von Kaulla, K. N.: Tromexan, ein neues Antikoagulans. Klinische Ergebnisse. Deutsche med. Wchnschr., **74**: 1259–1262, 1950.

270. von Kaulla, K. N. and Husemann, E.: Beitrag zur Synthese heparinähnlicher Körper. Zeitschr. f. d. ges. exper. Med., **114**: 722–732, 1945.

271. von Kaulla, K. N. and Pulver, R.: Tierexperimentelle Untersuchungen mit dem neuen Antithrombotikum Tromexan. Schweiz. med. Wchnschr., **78**: 806–820, 1948.

272. Kay, J. H. and Balla, G. A.: Antithromboplastic activity of monosodium alpha tocopherol phosphate and inositol phosphatide. Proc. Soc. Exper. Biol. & Med., **73**: 465–467, 1950.

273. Kay, J. H. and Hutton, S. B.: Studies on an antithrombin. I. The antithrombic activity of alpha tocopherol phosphate. Bull. Tulane Medical Faculty, **9**: 122–126, 1950.

274. Kay, J. H., Balla, G., Hutton, S. B. and Ochsner, A.: The prophylaxis of intravascular clotting by the use of alpha tocopherol and calcium. New Orleans M. & S. J., **103**: 116–120, 1950.

275. Kay, J. H., Hutton, S. B., Jr., Weiss, G. N. and Ochsner, A.: Studies on an antithrombin. III. A plasma antithrombin test for the prediction of intravascular clotting. Surgery, **28**: 24–28, 1950.

276. Kern, R. A. and Taugner, P. H., Jr.: Protamine and allergy. I. Nature of the local reactions after injections of protamine zinc insulin. II. Induction of sensitivity to insulin by injections of protamine zinc insulin. J. Am. M. A., **113**: 198–200, 1939.

277. de Keyser, R.: Nieuwe perspectieven in de antistolingstherapie. Geneeskundige Gids, **27**: 223–229, 1949.

278. Klein, P. D. and Seegers, W. H.: The nature of plasma antithrombin activity. Blood, **5**: 742–752, 1950.

279. Knisley, M. H.: Personal communication.

280. Knottenbelt, N.: Stollingremmende Behandeling von Thrombose. Thesis, University of Leiden, 1947.

281. Koller, V. F.: Die prophylaxe und therapie der thrombose mit antikoagulantien. Helv. med. Acta, **16**: 184–217, 1949.

282. Koller, F.: Ist die Anticoagulantientherapie der Thromboembolie ausserhalb des Spitals möglich? Praxis, Schweiz. Rundschau für Med., **39**: 68–71, 1950.

283. Kossel, A.: The protamines and histones, Longmans, Green and Co., 1928.

284 Kraus, A. P., Perlow, S. and Singer, K.: Danger of Dicumarol treatment in pregnancy. J. Am. M. A., **139**: 758–762, 1949.

285. Laufman, H. and Heller, R.: Effect of heparin on wound healing. Surg., Gynec. & Obst., **76**: 655–658, 1943.

286. Laufman, H., Martin, W. B. and Tanturi, C.: Effect of heparin and Dicoumarol on sludge formation. Science, **108**: 283–284, 1948.

287. Lee, C. C., Trevoy, L. W., Jaques, L. B. and Spinks, J. W. T.: The synthesis of 3,3'-methylene-C^{14}-bis (4-hydroxycoumarin). Canad. J. Research, B, **28**: 170–171, 1950.

288. Lee, C. C., Trevoy, L. W., Spinks, J. W. T. and Jaques, L. B.: Dicumarol labelled with C^{14}. Proc. Soc. Exper. Biol. & Med., **74**: 151–155, 1950.

289. Lehmann, J.: Effect of coumarin and dicoumarin derivatives on prothrombin level. Lancet, **1**: 458–459, 1943.

290. Lein, J. and Lein, P. S.: An altered prothrombin produced by Dicumarol-treated rabbits. Am. J. Physiol., **155**: 394–401, 1948.

291. Le Roy, G. V. and Nalefski, L. A.: Dicumarol in experimental myocardial infarction. J. Lab. & Clin. Med., **33**: 961–967, 1948.

292. Lester, O.: Non-appearance of salicylates in urine after administration of Dicoumarol. J. Biol. Chem., **154**: 305–306, 1944.

293. Levin, W. C. and Ruskin, A.: Studies on the effects of digitoxin on the coagulation mechanism. Am. J. Med., **7**: 133. 1949.

294. Levy, S. and Conroy, L.: Prothrombin time and anesthesia: A clinical investigation of the effects of ether and spinal anesthesia on the prothrombin level of the blood. Anesthesiology, **7**: 276–284, 1946.

295. Lewis, J. H.: Effect of penicillin on blood coagulation. Proc. Soc. Exper. Biol. & Med., **63**: 538–540, 1946.

296. Lewitus, Z. A.: Prothrombinopenic effect of penicillin. Arch. Int. Med., **82**: 625, 1948.

297. Lian, M. M. C., Siguier, F., Welti, J. J., Piette, M., Coblentz, B. and Trelat, J.: Reflexions sur un nouvel

anticoagulant: l'ester ethylique de l'acide d-3,3' (4-oxycoumarine) acetique. Societe Medicale des Hopitaux de Paris, **22:** 110-121, 1950.

298. Light, R. U.: The antigenicity of bovine thrombin: Clinical evaluation. J. Neurosurg., **2:** 516-523, 1945.
299. Light, R., Alscher, R. P. and Frey, C. N.: Vitamin A toxicity and hypoprothrombinemia. Science, **100:** 225-226, 1944.
300. Light, R. U. and Prentice H. R. Gelatin sponge. Surgical investigation of a new matrix used in conjunction with thrombin in hemostasis. Arch. Surg., **51:** 69-77, 1945.
301. Light, R. U. and Prentice, H. R.: Surgical investigation of a new absorbable sponge derived from gelatin for use in hemostasis. J. Neurosurg., **2:** 435-455, 1945.
302. Lilly, G. D. and Lee, R. M.: Complications of anticoagulant therapy. Surgery, **26:** 957-969, 1949.
303. Link, K. P.: The anticoagulant from spoiled sweet clover hay. Harvey Lect., **39:** 162-216, 1943-44.
304. Link, K. P.: The anticoagulant Dicumarol. Proc. Inst. Med. Chicago, **15:** 370-389, 1945.
305. Link, K. P.: The anticoagulant 3,3'-methylenebis (4-hydroxycoumarin). Federation Proc., **4:** 176-182, 1945.
306. Link, K. P.: The anticoagulant Dicumarol. Chicago Med. Soc. Bull., **51:** 57-62, 1948.
307. Link, K. P.: The hypoprothrombinemic action of salicylates. Chicago Med. Soc. Bull., **51:** 53-56, 1948.
308. Link, K. P., Overman, R. S., Sullivan, W. R., Huebner, C. F. and Scheel, L. D.: Studies on the hemorrhagic sweet clover disease. XI. Hypoprothrombinemia in the rat induced by salicylic acid. J. Biol. Chem., **147:** 463-474, 1943.
309. Linman, J. W., Duff, I. F. and Serfontein, S.: Anticoagulant therapy with depository heparin. Univ. Michigan M. Bull., **16:** 69-73, 1950.
310. Litwins, J., Vesell, H., Kissin, M., Cohen, I. and Paul, A.: Effect of Dicoumarol on the erythrocyte sedimentation rate. J. Am. M. A., **141:** 330, 1949.
311. Loewe, L. and Eiber, H. B.: Anticoagulation therapy with heparin/Pitkin menstruum in the management of coronary artery thrombosis and its complications. Am. Heart J., **37:** 701-719, 1949.
312. Loewe, L. and Hirsch, E.: Heparin in the treatment of thromboembolic disease. J. Am. M. A., **133:** 1263-1268, 1947.
313. Loewe, L. and Rosenblatt, P.: A new practical method for subcutaneous administration of heparin. Preliminary report. Am. J. M. Sc., **208:** 54-63, 1944.
314. Loewe, L., Rosenblatt, P. and Hirsch, E.: Venous thromboembolic disease. J. Am. M. A., **130:** 386-393, 1946.
315. Long, P. H.: Antibiotics and blood coagulation. J. Am. M. A., **142:** 49-50, 1950.
316. Lord, J. W., Jr.: Effect of trauma to the liver on the plasma prothrombin. Surgery, **6:** 896-898, 1939.
317. Losser, R. P., Versakos, M. and Loewe, L.: The influence of aureomycin on human coagulability. Angiology, **1:** 233-235, 1950.
318. Lucia, S. P. and Aggeler, P. M.: Treatment of Dicoumarol-induced hypoprothrombinemic hemorrhage with vitamin K_1 oxide. Proc. Soc. Exper. Biol. & Med., **56:** 36, 1944.
319. Lupton, A. M.: The effect of perfusion through the isolated liver on the prothrombin activity of blood from normal and Dicumarol treated rats. J. Pharmacol. & Exper. Therap., **89:** 306-312, 1947.
320. Lüscher, E.: Untersuchungen über einen die Blutgerinnung beschleunigenden Stoff aus dem Harne Hämophiler. Thesis, University of Bern, 1947.
321. Macfarlane, R. G.: Critical review: The mechanism of haemostasis. Quart. J. Med., **10:** 1-32, 1941.
322. Macfarlane, R. G.: Normal and abnormal blood coagulation: Review. J. Clin. Path., **1:** 113-143, 1948.
323. Macht, D. I.: Experimental studies on heparin and its influence on toxicity of digitaloids, congo red, cobra venom and other drugs. Ann. Int. Med., **18:** 772-791, 1943.
324. Macht, D. I.: Thromboplastic properties of some mercurial diuretics. Am. Heart J., **31:** 460-463, 1946.
325. Macht, D. I.: Thromboplastic properties of penicillin and streptomycin. Arch. internat. de pharmacodyn., et de thérap., **74:** 399-405, 1947.
326. Macht, D. I.: Thromboplastic properties of penicillin and streptomycin. Science, **105:** 313-314, 1947.
327. Macht, D. I.: Penicillin, streptomycin, Dicoumarol and blood coagulation. Thromboplastic properties of penicillin. South. M. J., **41:** 720-727, 1948.
328. Macht, D. I. and Farkas, R.: Aureomycin and blood coagulation. Science, **110:** 305-306, 1949.
329. MacLean, A. L. and Brambel, C. E.: Dicumarol and rutin in retinal vascular disorders. Trans. Am. Opthalmological Soc., 1946.
330. MacMillan, R. L.: Observations on the mechanism of action of Dicoumarol. Science, **108:** 416-417, 1948.
331. Mann, F. D. and Hurn, M. M.: The complex mechanisms of the Quick prothrombin test and the effect of Dicumarol. Am. J. Clin. Path., **20:** 225-232, 1950.
332. Mann, F. D., Hurn, M. and Magath, T. B.: Observation on the conversion of prothrombin to thrombin. Proc. Soc. Exper. Biol. & Med., **66:** 33-35, 1947.
333. Marple, C. D.: Anticoagulant therapy in heart disease. California Med., **73:** 425-431, 1950.
334. Marple, C. D.: The administration of anticoagulants. California Med., **73:** 3-7, 1950.
335. Marple, C. D. and Wright, I. S.: Thromboembolic conditions and their treatment with anticoagulants, C. C Thomas, 1950.
336. Mason, K. E.: A hemorrhagic state in the vitamin E-deficient fetus of the rat. Essays in Biology, 401-407, 1943.
337. McCall, M., Spence, J. and Coates, G.: Dicumarol therapy in acute coronary occlusion with myocardial infarction. Am. J. M. Sc., **215:** 612-616, 1948.
338. McCann, J. C.: Thromboembolism; A comparison of the effect of early postoperative ambulation and Dicumarol on its incidence. New England J. Med., **242:** 203-207, 1950.
339. McClaughry, R. I. and Seegers, W. H.: Prothrombin, thromboplastin, Ac-globulin and platelet accelerator: Quantitative interrelationships. Blood, **5:** 303-312, 1950.

340. McClaughry, R. I., Andrews, E. B. and Seegers, W. H.: Changes in the reactivity of purified prothrombin after freeze drying. Proc. Soc. Exper. Biol. & Med., **75:** 252–254, 1950.

341. McCleery, R. S., Artz, C. P. and Sirak, H. D.: Practical application of the heparin-caronamide reaction to the potentiation of heparin in gelatin menstruum. Surgery. **24:** 348–354, 1948.

342. McCormick, H. M. and Young, I. I.: Effect of aminophylline on plasma prothrombin and Ac-globulin in dogs. Proc. Soc. Exper. Biol. & Med., **70:** 501–503, 1949.

343. McGinty, D. A., Seegers, W. H., Pfeiffer, C. C. and Loew, E. R.: Plasma prothrombin concentration in dogs given 3,3′-methylenebis (4-hydroxycoumarin) and purified beef prothrombin. Science, **96:** 540–542, 1942.

344. Meissner, R.: Zur Beschleunigung der Blutgerinnung durch Euphyllin. Biochem. Ztschr., **120:** 197–202, 1921.

345. Mellanby, J.: The coagulation of blood. Part II. The actions of snake venoms, peptone, and leech extract. J. Physiol., **38:** 441–503, 1909.

346. Mendenhall, W.: Factors affecting the coagulation time of blood. VII. The influence of certain anesthetics. Am. J. Physiol., **38:** 33–51, 1915.

347. Menghini, G.: Anticoagulant action *in vitro* of sodium penicillin. Bull. Soc. ital. biol. sper., **24:** 720–721, 1948.

348. Mentzer, C.: The various groups of synthetic compounds with anti-vitamin K activity and the biological significance of results obtained (in animals). Bull. soc. chim. biol., **30:** 872–884, 1948.

349. Mentzer, C., Meunier, P., Lecocq, J., Billet, D. and Xuong, D.: Chemical studies of the antivitamin K series of compounds. Bull. soc. chim. biol., **12:** 430–437, 1945.

350. Meunier, P.: Mechanism of the action of antivitaminosis K in animals. Bull. soc. chim. biol., **30:** 884–891, 1948.

351. Meunier, P. and Mentzer, C.: Antihemorrhagic activity of certain chroman derivatives and the notion of antivitamin K. Bull. soc. chim. biol., **25:** 80–87, 1943.

352. Meunier, P., Mentzer, C. and Lajudie, J.: Antivitamins K (IV) Hemorrhagic activity of the salicylic acid series. Bull. soc. chim. biol., **29:** 977–981, 1947.

353. Meunier, P., Mentzer, C. and Molho, D.: Antivitamin K hemorrhagic activity of an indandione. Compt. rend. Acad. d. sc., **224:** 1666-1667, 1947.

354. Meunier, P., Mentzer, C. and Vinet, A.: Monocoumarin derivatives with antivitamin K activity. Helv. chim. Acta, **29:** 1291–1297, 1946.

355. Meyer, O. and Howard, B.: Production of hypoprothrombinemia and hypocoagulability of the blood with salicylates. Proc. Soc. Exper. Biol. & Med., **53:** 234–237, 1943.

356. Milhet, M. F.: La thromboplastine et ses troubles, Imprimerie des Beaux-Arts, 1949.

357. Miller, J. A. W. and Drucquer, J. K.: Case of Dicumarol poisoning. Brit. M. J., **2:** 904, 1948.

358. Miller R., Harvey, W. P. and Finch, C. A.: Antagonism of Dicumarol by vitamin K preparations. New England J. Med., **242:** 211–215, 1950.

359. Mills, C. A.: Chemical nature of tissue coagulins. J. Biol. Chem., **46:** 135, 1921.

360. Milstone, J. H.: Activation of prothrombin by platelets plus globulin. Proc. Soc. Exper. Biol. & Med., **68:** 225–228, 1948.

361. Moldavsky, L. F., Hasselbrook, W. B. and Cateno, C.: Studies in mechanisms of penicillin action. I. Penicillin effects on blood coagulation. Science, **102:** 38–40, 1945.

362. Molho, D., Moraux, J. and Meunier, P.: Antivitamins K. V. Hemorrhagic activity in rabbits of 2-methoxy-1,4-naphthoquinone and related compounds. Bull. soc. chim. biol., **30:** 637–642, 1948.

363. Moloney, W. C., Egan, W. J. and Gorman, A. J.: Acquired afibrinogenemia in pregnancy. New England J. Med., **240:** 596–598, 1949.

364. Moses, C.: Effect of digitalis, epinephrine and surgery on the response to heparin. J. Lab. & Clin. Med., **30:** 603–607, 1945.

365. Mosonyi, L., Palos, L. A. and Komaromy, J.: Penicillin and blood coagulation. Orvosi Hetilap, **89:** 545–550, 1948.

366. Mosonyi, L., Palos, L. A. and Komaromy, J.: Penicillin and blood coagulation. Acta med. Scandinav., **135:** 458–464, 1949.

367. Moss, N. H., Schafer, R. L. and Kirby, C. K.: Anticoagulant effect of Dicoumarol at various prothrombin levels in dogs. Proc. Soc. Exper. Biol. & Med., **69:** 143–145, 1948.

368. Muir, J. D.: Activity of heparin in Pitkin's menstruum. Lancet, **2:** 671–672, 1950.

369. Mulford, D. J.: Derivatives of blood plasma. Ann. Rev. Physiol., **9:** 327–356, 1947.

370. Munro, F. L. and Lupton, A. M.: The influence of Dicumarol treatment of rabbits, rats and dogs on the thromboplastic activity of their brain suspensions. Arch. Biochem., **15:** 245–250, 1947.

371. von Muralt, G. and Hirsiger, W.: Prothrombin level of human blood on high mountains. Helv. physiol. et Pharmacol. Acta, **6:** 626–638, 1948.

372. Murray, G. D. W. and Best, C. H.: Use of heparin in thrombosis. Ann. Surg. **108:** 163–177, 1938.

373. Murray, D. W. G., Jaques, L. B., Perrett, T. S. and Best, C. H.: Heparin and vascular occlusion. Canad. M. A. J., **35:** 621–622, 1936.

374. Murray, D. W. G., Jaques, L. B., Perrett, T. S. and Best, C. H.: Heparin and the thrombosis of veins following injury. Surgery, **2:** 163–187, 1937.

375. Mushett, C. W. and Seeler, A. O.: Hypoprothrombinemia resulting from the administration of sulfaquinoxaline. J. Pharmacol. & Exper. Therap., **91:** 84–91, 1947.

376. Mylon, E., Winternitz, M. C. and de Sütő-Nagy, G. J.: The determination of fibrinogen with protamine. J. Biol. Chem., **143:** 21–27, 1942.

377. Nanninga, L. B.: Investigations on fibrinogen and thrombin, the second phase of blood coagulation. Thesis, University of Amsterdam, 1947.

378. Nassi, L. and Ulivelli, A.: In vitro action of streptomycin on the blood coagulation time. Boll. Soc. ital. biol. sper., **24:** 956–958, 1948.

379. Neivert, H.: Late secondary tonsillar hemorrhage. I. Studies on prothrombin and vitamin K. Arch. Otolaryng., **42:** 14–18, 1945.

380. Nichol, E. S.: The use of anticoagulants in acute coronary insufficiency or impending myocardial infarction. South. M. J., **43:** 565–574, 1950.

381. de Nicola, P.: L'estere etilico dell'acido di (4-ossicumaril) acetico e la caratteristiche biologische della sua attivita anticoagulante. Il Farmaco, **4:** 3–11, 1949.

382. de Nicola, P. and Rosti, P.: Sulle fibrinogenopenie e ipoprotrombemie sperimentali da infusione di trombina. Boll. Soc. ital. biol. sper., **25:** 8–12, 1949.

383. Nitshe, G. A., Jr., Gerarde, H. W. and Deutsche, H. F.: Prothrombin-time studies on human beings and experimental animals. J. Lab. & Clin. Med., **32:** 410–418, 1947.

384. Nolf, P.: The coagulation of the blood. Medicine, **17:** 381–411, 1938.

385. Nonnenbruck, W. and Szyska, W.: Ueber einige neuartige Mittel (Euphyllin und andere Amine) zur Beschleunigung der Blutgerinnung. Deutsches Arch. f. klin. Med., **134:** 174–184, 1920.

386. Norris, R. F. and Bennett, M. C.: Plasma prothrombin values of mothers and infants at delivery: Further studies including comparative values of the umbilical arteries and veins. Surg., Gynec. & Obst., **72:** 758–763, 1941.

387. Nygaard, K. K.: Hemorrhagic diseases, Mosby, 1941.

388. Obata, I.: On the nature of eclampsia. J. Immunol., **4:** 111–139, 1919.

389 Olwin, J. H.: The control of Dicumarol therapy. Am. J. M. Sc., **217:** 427–437, 1949.

390. Overman, R. S. and Wright, I. S.: A new clotting inhibitor. J. Biol. Chem., **174:** 759–760, 1948.

391. Overman, R. S. and Wright, I. S.: The effect of aminophyllin on the prothrombin time of normal human plasma Am. Heart J., **39:** 65–68, 1950.

392. Overman, R. S. and Wright, I. S.: Prothrombin time determinations on patients with myocardial infarction. J. Am. M. A., in press.

393. Overman, R. S., Sorenson, C. W. and Wright, I. S.: The effectiveness of synthetic water soluble vitamin K preparations in Dicumarol induced hypoprothrombinemia. J. Am. M. A., **145:** 393–399, 1951.

394. Overman, R. S., Stahmann, M. A., Huebner, C. F., Sullivan, W. R., Spero, L., Doherty, D. G., Ikawa, M., Graf, L., Roseman, S. and Link, K. P.: Studies on the hemorrhagic sweet clover disease. XIV. Anticoagulant activity and structure in the 4-hydroxycoumarin group. J. Biol. Chem., **153:** 5–24, 1944.

395. Owen, C. A. and Bollman, J. L.: Prothrombin conversion factor of Dicoumarol plasma. Proc. Soc. Exper. Biol. & Med., **67:** 231–234, 1948.

396. Owen, C. A., Hoffman, G. R., Ziffren, S. E. and Smith, H. P.: Blood coagulation during infancy. Proc. Soc. Exper. Biol. & Med., **41:** 181–185, 1939.

397. Owen, G. C. and Bradford, H. A.: The prothrombinopenic effect of massive salicylate therapy in acute rheumatic fever. Ann. Int. Med., **25:** 97–102, 1946.

398. Owren, P. A.: The coagulation of blood. Acta med. Scandinav., Suppl. No. 194, 1947.

399. Owren, P. A.: A new, previously unknown clotting factor. Scandinav. J. Clin. & Lab. Invest., **1:** 162, 1949.

400. Owren, P. A.: The diagnostic and prognostic significance of plasma prothrombin and factor V levels in parenchymatous hepatitis and obstructive jaundice. Scandinav. J. Lab. & Clin. Med., **1:** 131–140, 1949.

401. Owren, P. A.: Acta med. Scandinav., 2nd Congress of International Medicine, 1950, in press.

402. Page, E. W., Fulton, L. D. and Glendening, M. B.: The cause of the blood coagulation defect following abruptio placentae. Am. J. Obst. & Gynec., in press.

403. Palmer, J. D. and Gundersen, S. M.: The effect of Dicumarol on the erythrocyte sedimentation rate in normal man. New England J. Med., **239:** 818–819, 1948.

404. Palos, L. A.: Oxygen metabolism and thrombin activation. Schweiz. med. Wchnschr., **78:** 112–115, 1948.

405. Palos, L. A.: Respiration and blood coagulation. Acta med. Scandinav., **134:** 221–224, 1949.

406. Pappenheimer, A. M. and Goettsch, M.: A cerebellar disorder in chicks, apparently of nutritional origin. J. Exper. Med., **53:** 11–26, 1931.

407. Parkin, T. W. and Kvale, W. F.: Neutralization of anticoagulant effects of heparin with protamine (Salmine). Am. Heart J., **37:** 333–342, 1949.

408. Parkin, T. W., Hall, B. E. and Watkins, C. H.: Experience with antiheparin compounds in essential thrombocytopenic purpura. Proc. Staff Meet., Mayo Clin., **23:** 309–316, 1948.

409. Parry, T. G. W. and Laszlo, G. C.: Thrombin technique in ophthalmic surgery. Brit. J. Ophth., **30:** 176–178, 1946.

410. Patton, T. B., Ware, A. G. and Seegers, W. H.: Clotting of plasma and silicone surfaces. Blood, **3:** 656–659, 1948.

411. Pere, S. A. N.: The effect of digitalis, strophanthin, and novurit on blood coagulation. Thesis, University of Helsinki, 1950.

412. Permin, P. M.: Undersøgelser over fibrinolytiske enzymer, Store Nordiske Videnskabsboghandel, 1949.

413. Peters, H. R., Doenges, J. P. and Brambel, C. E.: Further experiences with Dicumarol therapy in coronary thrombosis. South. M. J., **41:** 526–534, 1948.

414. Peters, H. R., Guttner, J. R. and Brambel, C. E.: Dicumarol in acute coronary thrombosis. J. Am. M. A., **130:** 398–403, 1946.

415. Phelps, E. T.: The dangers of Dicumarol therapy. M. Clin. North America, **34:** 1791–1800, 1950.

416. Pickering, J. W.: The blood plasma in health and disease, Macmillan, 1928.

417. Pilcher, C. and Meacham, W. F.: Absorbable gelatin sponge and thrombin for hemostasis in neurosurgery: Experimental and clinical observations. Surg., Gynec. & Obst., **81**: 365–369, 1945.

418. Piper, J.: Influence of synthetic polysaccharid sulfuric acid esters on the thrombocytes in vivo and in vitro. Acta physiol. Scandinav., **9**: 28–38, 1945.

419. Piper, J.: The anticoagulant effect of heparin and synthetic polysulphuric acid esters. Acta pharmacol. et toxicol., **2**: 138–148, 1946.

420. Piper, J.: Toxicology of synthetic polysaccharide polysulphuric acid esters. Acta pharmacol. et toxicol., **2**: 317–328, 1946.

421. Piper, J.: The fate of heparin in rabbits after intravenous injection. Filtration and tubular secretion in the kidneys. Acta pharmacol. et toxicol., **3**: 373–384, 1947.

422. Ponka, J. L. and Lam, C. R.: Effect of asphyxia on blood coagulation in dogs. Proc. Soc. Exper. Biol. & Med., **68**: 334–336, 1948.

423. Prandoni, A. and Wright, I.: The anti-coagulants. Heparin and the dicoumarin-3,3′-methylene-bis (4-hydroxy-coumarin). Bull. New York Acad. Med., **18**: 433–458, 1942.

424. Puharich, H. K. and Goetzl, F. R.: Influence of digitalis and other digitaloid drugs on blood coagulation. Permanente Found. (Oakland, Calif.), M. Bull., **5**: 97–110, 1947.

425. Pulaski, E. J., Reichel, H. and Voorhees, A. B., Jr.: Effects of adrenoxyl on blood coagulation mechanism and vasomotor response. Proc. Soc. Exper. Biol. & Med., **70**: 504–507, 1949.

426. Pulver, R. and von Kaulla, K. N.: Ueber Resorption und biologische Inaktivierung des neuen Antithromboticums Tromexan. Schweiz. med. Wchnschr., **78**: 956–965, 1948.

427. Putnam, T. J.: The use of thrombin on soluble cellulose in neurosurgery. Clinical application. Ann. Surg., **118**: 127–129, 1943.

428. Quastel, J. H. and Recker, E.: Tissue anoxia and blood coagulation. Brit. J. Exper. Path., **22**: 15–17, 1941.

429. Quick, A. J.: Is heparin an antiprothrombin? Proc. Soc. Exper. Biol. & Med., **35**: 391–392, 1936.

430. Quick, A. J.: The coagulation defect in sweet clover disease and in the hemorrhagic chick disease of dietary origin. Am. J. Physiol., **118**: 260–271, 1937.

431. Quick, A. J.: The normal antithrombin of the blood and its relation to heparin. Am. J. Physiol., **123**: 712–719, 1938.

432. Quick, A. J.: The hemorrhagic diseases, C. C Thomas and Co., 1942.

433. Quick, A. J.: The anticoagulants effective in vivo with special reference to heparin and Dicumarol. Physiol. Rev., **24**: 297–318, 1944.

434. Quick, A. J.: Experimentally induced changes in the prothrombin level of the blood. I. Quantitative studies in dogs given Dicumarol. II. The effect of methylxanthines on prothrombin per se and when administered with Dicumarol. J. Biol. Chem., **161**: 33–44, 1945.

435. Quick, A. J.: On the quantitative estimation of prothrombin. Am. J. Clin. Path., **15**: 560–566, 1945.

436. Quick, A. J.: Experimentally induced changes in the prothrombin level of the blood. III. Prothrombin concentration of new-born pups of a mother given Dicumarol before parturition. J. Biol. Chem., **164**: 371–376, 1946.

437. Quick, A. J.: Antibiotics and blood coagulation. J. Am. M. A., **142**: 505, 1950.

438. Quick, A. J. and Stefanini, M.: Experimentally induced changes in the prothrombin level of the blood. IV. The relation of vitamin K deficiency to the intensity of Dicoumarol action and to the effect of excess vitamin A intake; with a simplified method for vitamin K assay. J. Biol. Chem., **175**: 945–952, 1948.

439. Quick, A. J., Shanberge, J. N. and Stefanini, M.: The effect of heparin on platelets in vivo. J. Lab. & Clin. Med., **33**: 1424–1430, 1948.

440. Quinby, W. C.: Hemostasis by thrombin and fibrin foam in suprapubic prostatectomy: An analysis of 40 cases. New England J. Med., **235**: 609–612, 1946.

441. Ramsey, H., Pinschmidt, N. W. and Haag, H. B.: The effect of digitalis upon coagulation time of the blood. J. Pharmacol. & Exper. Therap., **85**: 159–169, 1945.

442. Rapoport, S., Wing, M. and Guest, G. M.: Hypoprothrombinemia after salicylate administration in man and rabbits. Proc. Soc. Exper. Biol. & Med., **53**: 40–41, 1943.

443. Ratcliff, R. K. and Plumb, R. T.: The use of thrombin in the control of postprostatic resection blood loss. Urol. & Cutan. Rev., **50**: 199–201, 1946.

444. Ravdin, I. S. and Johnston, C. G.: Heparin. Am. J. M. Sc., **201**: 299–302, 1941.

445. Reading, P.: Some uses of thrombin in the surgery of the ear, nose, and throat. Brit. M. J., **2**: 295–296, 1946.

446. Reiner, L., de Beer, E. J. and Green, M.: Toxic effects of some basic proteins. Proc. Soc. Exper. Biol. & Med., **50**: 70–74, 1942.

447. von Reinis, Z. and Kubik, M.: Klinische Erfahrungen mit einem neuen Präparat der Cumarinreihe. Schweiz. med. Wchnschr., **78**: 785–790, 1948.

448. Richards, R. K.: Influence of fever upon the action of 3,3′-methylenebis-(4-hydroxy-coumarin) (Dicumarol). Science, **97**: 313, 1943.

449. Richards, R. K. and Steggerda, F. R.: Dicumarol (3,3′-methylenebis-(4-hydroxy-coumarin)) in rats with impaired liver or kidney function. Proc. Soc. Exper. Biol. & Med., **52**: 358–360, 1943.

450. Richter, I. H., Eiber, H. B. and Loewe, L.: Subcutaneous heparin in the treatment of arterial thrombolic diseases. Preliminary report. Surgery, **22**: 489–495, 1947.

451. Rieben, W. K.: Beobachtungen mit einer neuen Prothrombinbestimmungsmethode. Helvet. med. Acta, **13**: 295–303, 1946.

452. Riggs, D. S.: Anticoagulants. New England J. Med., **242**: 179–184, 1950.

453. Roblin, R. O., Jr.: Metabolite antagonists. Chem. Rev., **38:** 255–377, 1946.

454. Rogers, T. M.: Management of gastric hemorrhage using topical thrombin. J. Am. M. A., **137:** 1035–1036, 1948.

454a. Rohwer, S. A. U. S. D. A. Interdepartmental Comm. Pest Control, June 29, 1950. Warfarin, a coined name for the anticoagulant rodenticidal chemical 3-(α-acetonylbenzyl)-4-hydroxycoumarin with an attachment on the chemistry and physiological actions prepared by Karl Paul Link 1/20/1950.

455. Rose, C. L., Harris, P. N. and Chen, K. K.: Toxicity of 3,3′-methylenebis (4-hydroxycoumarin). Proc. Soc. Exper. Biol. & Med., **50:** 228–232, 1942.

456. Rosenfield, R. E. and Tuft, H. S.: Preliminary observations on the adsorption of prothrombin by barium sulfate. Arch. Biochem., **16:** 329–332, 1948.

457. Roskam, J. and Derouaux, G.: Hémostase spontanée et réactivite adrénergique. Arch. internat. de pharmacodyn. et de thérap., **69:** 12, 1943.

458. Roskam, J., Derouaux, G., Mets, L. and Swalue, L.: Un nouvel hémostatique biologique: la monosemicarbazone d'adrénochrome où adrénoxyl. Arch. internat. de pharmacodyn. et de thérap., **74:** 162–175, 1947.

459. Ross, S., Burke, F. G., Rice, E. C., Schoenbach, E. B., Bischoff, H. and Washington, J. A.: Aureomycin: Preliminary report of a clinical trial. Clin. Proc. Child. Hosp., **4:** 315–331, 1948.

460. Rosti, P.: Action of Dicumarol on thromboplastic activity of the rabbit brain. Boll. ist. sieroterap milan, **27:** 122–128, 1948.

461. Sah, P. P. T.: Synthesis of 3-methyl-4-amino-1-naphthol hydrochloride (vitamin K_7) and related vitamin-K-active compounds. Hormon. u. Formentforsch., **3:** 324–345, 1949–50.

462. Sakurai, K.: Studies on toxicity of human placenta upon rabbits and its accelerating influence upon coagulation of blood, and on its chemical nature. Sei-I-Kwai M. J., **48:** 4–6, 1929.

463. Salvini, M., Feruglio, F. S. and Miani, P.: Anticoagulants and lactation. Boll. Soc. ital. biol. sper., **24:** 991–993, 1948.

463a. Scheel, L. D.: Doctoral Dissertation, University of Wisconsin, June, 1949.

464. Schenker, N. P.: Thrombosis and embolism. Motion picture, The international and Fourth American Congress on Obst. & Gynec., N. Y., 1950.

465. Scherf, D. and Schlachman, M.: The effect of methylxanthines on the prothrombin time and the coagulation of the blood. Am. J. M. Sc., **212:** 83–89, 1946.

466. Schmorl, G.: Pathologisch anatomische Untersuchungen über Puerpural Eklampsie, F. C. W. Vogel, 1893.

467. Schneider, C. L.: Desensitization of mice to placental toxin. Am. J. Physiol., **147:** 250–254, 1946.

468. Schneider, C. L.: Inactivation of placental toxin by human serum. Am. J. Physiol., **146:** 140–145, 1946.

469. Schneider, C. L.: Liver necrosis in mice following injection of toxic extracts of progestational endometrium and of placenta. Proc. Soc. Exper. Biol. & Med., **62:** 325–328, 1946.

470. Schneider, C. L.: Placental toxin: Inactivation and tolerance during pregnancy. Am. J. Physiol., **147:** 255–259, 1946.

471. Schneider, C. L.: Toxic principle from progestational endometrium and placenta. Proc. Soc. Exper. Biol. & Med., **62:** 322–325, 1946.

472. Schneider, C. L.: Active principle of placental toxin: Thromboplastin; its inactivator in blood: Antithromboplastin. Am. J. Physiol., **149:** 123–129, 1947.

473. Schneider, C. L.: Complications of late pregnancy in rabbits induced by experimental placental trauma. Surg., Gynec. & Obst., **90:** 613–622, 1950.

474. Schneider, C. L.: Thromboplastin complications of late pregnancy. Ciba Foundation Symposium on Toxemia of Pregnancy in animals and in woman. J. and A. Churchill, 163–181, 1950.

475. Schneider, C. L.: "Fibrin embolism" (disseminated intravascular coagulation) with defibrination as one of the end results, during placenta abruptio. Surg., Gynec. & Obst., **92:** 27–32, 1951.

476. Schneider, C. L.: Thromboplastin complications of pregnancy. Bull. Margaret Hague Maternity Hosp., in press.

477. Schneider, C. L.: Relationship of fibrin embolism (disseminated intravascular coagulation) to the etiology of eclampsia. In preparation for the J. Obst. & Gynec., British Empire.

478. Schneider, C. L.: Unpublished data.

479. Schönholzer, G. and von Sinner, F.: The fibrinogen content of blood plasma on high mountains. Helv. physiol. et Pharmacol. Acta, **6:** 639–648, 1948.

480. Schultze, H. E.: Neuere Erkenntnisse über das Prothrombin und das Accelerator-globulin von Seegers. Deutsche med. Wchnschr., **75:** 607–611, 1950.

481. Searls, P. W.: The effect of certain anesthetics on the blood. J. Am. M. A., **113:** 906–909, 1939.

482. Searls, P. W. and Essex, H. E.: Changes in blood in course of ether anesthesia and sodium amytal anesthesia. Proc. Staff Meet., Mayo Clin., **11:** 481–483, 1936.

483. Seegers, W. H.: Purification of prothrombin and thrombin: Chemical properties of purified preparations. J. Biol. Chem., **136:** 103–111, 1940.

484. Seegers, W. H.: Activation of purified prothrombin. Proc. Soc. Exper. Biol. & Med., **72:** 677–680, 1949.

485. Seegers, W. H.: Blood coagulation and the practical significance of recent advances in knowledge of prothrombin and Ac-globulin. Circulation, **1:** 2–9, 1950.

486. Seegers, W. H.: Factors in the control of bleeding. Cincinnati J. Med., **31:** 395–402, 1950.

487. Seegers, W. H.: Blood coagulation. Chapter in The Enzymes, Vol. 2, Academic Press, Inc., 1951.

488. Seegers, W. H. and McClaughry, R. I.: Production of an inactive derivative of purified prothrombin by means of purified thrombin. Proc. Soc. Exper. Biol. & Med., **72:** 247–249, 1949.

489. Seegers, W. H. and McGinty, D. A.: Further purification of thrombin. Probable purity of products. J. Biol. Chem., **146:** 511–518, 1942.

490. Seegers, W. H. and Schneider, C. L.: The nature of the blood coagulation mechanism and its relationship to some unsolved problems in obstetrics and gynecology. Fourth International and American Congress on Obstetrics and Gynecology, New York, in press.
491. Seegers, W. H. and Sharp, E. A.: Hemostatic agents, C. C Thomas and Co., 1948.
492. Seegers, W. H. and Smith, H. P.: The purification of prothrombin. J. Biol. Chem., 140: 677–678, 1941.
493. Seegers, W. H., Andrews, E. B. and McClaughry, R. I.: Formation of prothrombin derivatives from purified prothrombin. Am. J. Physiol., 164: in press.
494. Seegers, W. H., Brinkhous, K. M., Smith, H. P. and Warner, E. D.: The purification of thrombin. J. Biol. Chem., 126: 91–95, 1938.
495. Seegers, W. H., Loomis, E. C. and Vandenbelt, J. M.: Preparation of prothrombin products: Isolation of prothrombin and its properties. Arch. Biochem., 6: 85–95, 1945.
496. Seegers, W. H., Warner, E. D., Brinkhous, K. M. and Smith, H. P.: The use of purified thrombin as an hemostatic agent. Science, 89: 86, 1939.
497. Seeler, A. O., Mushett, C. W., Graessle, O. and Silber, R. H.: Pharmacological studies on sulfaquinoxaline. J. Pharmacol. & Exper. Therap., 82: 357–363, 1944.
498. Seifter, J. and Begany, A. J.: Studies on the action of a synthetic heparinoid. Am. J. M. Sc., 216: 234–235. 1948.
499. Selander, P. and Bernius, B.: Das Prothrombin bei Säuglingen bei äusserer Salicylsäuerapplikation. Ann. Paediat., 169: 404–406, 1947.
500. Selye, H.: The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol., 6: 117–230, 1946.
501. Shapiro, S.: Studies on prothrombin. VI. Effect of synthetic vitamin K on prothrombinopenia induced by salicylate in man. J. Am. M. A., 125: 546–548, 1944.
502. Shapiro, S.: Anticoagulant therapy in the treatment of thrombosis. Hebrew M. J., June, 1947.
503. Shapiro, S. and Weiner, M.: Coagulation, thrombosis and Dicumarol, Brooklyn Medical Press, 1949.
504. Shapiro, S., Redish, M. H. and Campbell, H. A.: Prothrombin studies. III. Effect of vitamin K upon hypoprothrombinemia induced by Dicumarol in man. Proc. Soc. Exper. Biol. & Med., 52: 12–15, 1943.
505. Shapiro, S., Redish, M. H. and Campbell, H. A.: Studies on prothrombin. IV. The prothrombopenic effect of salicylates in man. Proc. Soc. Exper. Biol. & Med., 53: 251–254, 1943.
506. Shapiro, S., Wiener, M. and Simson, G.: The effect of water-soluble preparations of vitamin K in Dicumarol-induced hypoprothrombinemia. New England J. Med., 243: 775–779, 1950.
507. Shapse, J. B. and Wright, L. T.: Effect of aureomycin on the clotting time of the blood. Angiology, 1: 306–311, 1950.
508. Shelley, W. B., Hodgkins, M. P. and Visscher, M. B.: Studies on the toxicity of protamine. Proc. Soc. Exper. Biol. & Med., 50: 300–304, 1942.
509. Sherry, S., Johnson, A. and Tillett, W. S.: The action of streptococcal desoxyribose nuclease (streptodornase) *in vitro* and on purulent pleural exudations of patients. J. Clin. Investigation, 28: 1094–1104, 1949.
510. Sirasaka, T.: The influence of various pharmaceutical agents on the bleeding time and amount of bleeding. II. The action of some cardio-tonic drugs. Okayama-Igakkai-Zasshi (Mitt. med. Ges. Okayama), 53: 193–204, 1941.
511. Smith, C. C.: Antagonism of the hemorrhagic syndrome induced by derivatives of 3-hydroxyl-1,4-naphthoquinone. Proc. Soc. Exper. Biol. & Med., 64: 45–50, 1947.
512. Smith, C. C.: Absence of hemorrhagic inducing activity in phthiocol derivatives with a basic nitrogen in side chain. Proc. Soc. Exper. Biol. & Med., 73: 562–563, 1950.
513. Smith, C. C., Fradkin, R. and Lockey, M. D.: A hemorrhagic syndrome induced by derivatives of 3-hydroxy-1,4-naphthoquinone. Proc. Soc. Exper. Biol. & Med., 61: 398–403, 1946.
514. Smith, H. P.: Blood. Ann. Rev. Physiol., 4: 245–272, 1942.
515. Smith, H. P. and Flynn, J. E.: The coagulation of blood. Ann. Rev. Physiol., 10: 417–444, 1948.
516. Smith, H. P., Warner, E. D. and Brinkhous, K. M.: Prothrombin deficiency and the bleeding tendency in liver injury (chloroform intoxication). J. Exper. Med., 66: 801–811, 1937.
517. Smith, P. K.: Certain aspects of the pharmacology of the salicylates. Pharmacol. Rev., 1: 353–382, 1949.
518. Smith, R. W., Margulis, R. R., Brennan, M. J. and Monto, R. W.: The influence of ACTH and cortisone on certain factors of blood coagulation. Science, 112: 295–297, 1950.
519. Sobotka, H. and Adelman, N.: Shortening of bleeding time by a water soluble adrenochrome derivative. Proc. Soc. Exper. Biol. & Med., 75: 789–791, 1950.
520. Sokoloff, L. and Ferrer, M. J.: Effect of digitalization on the coagulation time in man. Proc. Soc. Exper. Biol. & Med., 59: 309–311, 1945.
521. Solandt, D. U. and Best, C. H.: Heparin and coronary thrombosis in experimental animals. Lancet, 2: 130–132, 1938.
522. Solomon, C., McNeile, H. J. and Lange, R.: Experience with the new anticoagulant, B. O. E. A. J. Lab. & Clin. Med., 36: 19–22, 1950.
523. Sorbye, O.: Demonstration of new accelerators in prothrombin activation. Proc. XVIII International Physiological Congress: 459–460, 1950.
524. Sorbye, O., Kruse, I. and Dam, H.: The factor in vitamin K-deficient plasma which accelerates the coagulation of Dicumarol plasma. Acta chem. Scandinav., 4: 549–550, 1950.
525. Sorenson, C. W. and Wright, I. S.: A synthetic anticoagulant: a polysulfuric acid ester of polyanhydromannuronic acid (paritol). Circulation, 2: 658–667, 1950.

526. Sorenson, C. W., Seifter, J. and Wright, I. S.: A new synthetic anticoagulant (heparinoid): Preliminary report of its action in humans. Bull. New York Acad. Med., **25**: 448, 1949.

527. Soulier, J. and Gueguen, J.: Action hypoprothrombinemiante (anti-K) de la phényl-indanedione étudiée experimentalement chez le lapin. Son application chez l'homme. Compt. rend. Soc. de biol., **141**: 1007–1111, 1947.

528. Soulier, J. P. and Koupernik, C.: Constant occurrence of fibrinolysis during acetylcholine induced shock Le Sang, **19**: 362–369, 1948.

529. Spinks, J. W. T. and Jaques, L. B.: Tracer experiments in mammals with Dicoumarol labelled with carbon^{14} Nature, **166**: 184, 1950.

530. Spooner, M. and Meyer, O. O.: The effect of Dicumarol (3,3′-methylenebis (4-hydroxycoumarin)) on platelet adhesiveness. Am. J. Physiol., **142**: 279–283, 1944.

531. Stahmann, M. A., Graf, L. H., Huebner, C. F., Roseman, S. and Link, K. P.: Studies on 4-hydroxycoumarins. IV. Esters of the 4-hydroxycoumarins. J. Am. Chem. Soc., **66**: 900–902, 1944.

532. Stahmann, M. A., Wolff, I. and Link, K. P.: Studies on 4-hydroxycoumarins. I. The synthesis of 4-hydroxycoumarins. J. Am. Chem. Soc., **65**: 2285–2287, 1943.

533. Stats, D. and Neuhof, H.: Concentrated heparin aqueous: New form of intramuscular injection. Am. J. M. Sc., **214**: 159–162, 1947.

534. Stefanini, M. and Blanchaer, M. C.: Action of Dicoumarol on thromboplastic activity of rabbit brain. Proc. Soc. Exper. Biol. & Med., **64**: 47–50, 1947.

535. Stefanini, M. and Petrillo, E.: Influence of some substances on the prothrombin time of human plasma in vitro. Boll. Soc. ital. biol. sper., **22**: 366–367, 1946.

536. Stefanini, M. and Pisciotta, A. V.: Rate of disappearance of prothrombin from the circulation. Science, **111**: 364, 1950.

537. Stevens, D. L. and Kaplan, D. B.: Salicylate intoxication in children: Report of 4 cases. Am. J. Dis. Child., **70**: 331–335, 1945.

538. Straith, C. L. and McEvitt, W. G.: Total avulsion of the scalp. Occup. Med., **1**: 451–462, 1946.

539. Strausz, I.: Effect of penicillin on blood clotting in vitro. Orv. Lapja, **3**: 73–74, 1948.

540. Sullivan, W. R., Gangstad, E. I. and Link, K. P.: Studies on the hemorrhagic sweet clover disease. XII. The effect of 1-ascorbic acid on the hypoprothrombinemia induced by 3,3′-methylenebis-(4-hydroxycoumarin) in the guinea pig. J. Biol. Chem., **151**: 477–485, 1943.

541. Sullivan, W. R., Huebner, C. F., Stahmann, M. A. and Link, K. P.: Studies on 4-hydroxycoumarins. II. The condensation of aldehydes with 4-hydroxycoumarins. J. Am. Chem. Soc., **65**: 2288–2291, 1943.

542. Supek, Z.: Digitalis compounds and blood coagulation. Arch. internat. de pharmacodyn. et de thérap., **77**: 175–185, 1948.

543. Surgenor, D. M., Alexander, B., Goldstein, R. and Schmid, K.: A system for the separation of the protein components of human plasma. II. J. Phys. & Coll. Chem., **55**: 94–101, 1951.

544. Sutton, G. C.: Studies on blood coagulation and the effect of digitalis. Circulation, **2**: 271–277, 1950.

545. von Sydow, G.: Hypoprothrombinemia and cerebral injury in a newborn infant after dicoumarin treatment of the mother. Nord. med., **34**: 1171–1172, 1947.

546. Sykes, E. M., Jr., Seegers, W. H. and Ware, A. G.: Effect of acute liver damage on Ac-globulin activity of plasma. Proc. Soc. Exper. Biol. & Med., **67**: 506–507, 1948.

547. Sylvén, B.: On the cytoplasmic constituents of normal tisque mast cells. VIIth International Congress International Society for Cell Biology, 49–50, 1950.

548. Sylvén, B.: Personal communication, August, 1950.

549. Tagnon, H. J.: The nature of the mechanism of the shock produced by the injection of trypsin and thrombin. J. Clin. Investigation, **24**: 1–10, 1945.

550. de Takats, G.: Heparin tolerance. A test of the clotting mechanism. Surg., Gynec. & Obst., **77**: 31–39, 1943.

551. de Takats, G.: Nervous regulation of clotting mechanism. Arch. Surg., **48**: 105–108, 1944.

552. ee Takats, G.: Anticoagulant therapy in surgery. J. Am. M. A., **142**: 527–533, 1950.

553. de Takats, G.: A sensitized clotting time. Angiology, **1**: 317–333, 1950.

554. de Takats, G., Trump, R. A. and Gilbert, N. C.: The effect of digitalis on the clotting mechanism. J. Am. M. A , **125**: 840–845, 1944.

555. Talmasse, V.: Modification of bleeding time after intravenous injection of thrombin. Compt. rend. Soc. de biol., **144**: 1265, 1950.

556. Taltavull, R. J., Vedove, A. D. and Maurelli, V. R.: Coagulation, bleeding and prothrombin time in patients receiving digitalis treatment. Revista Medica de Rosario, **36**: 36, 1946. Quoted by the J. Am. M. A., **131**: 871, 1946.

557. Tanaka, H.: Ueber den Einfluss des Strophanthins auf die Gerinnbarkheit des Blutes. Okayama-Igakkai-Zasshi, **40**: 1817, 1928.

558. Thayer, S. A.: The relationship of vitamin K to hemorrhage and coagulation. Ann. New York Acad. Sc., **49**: 518–524, 1948.

559. Thoma, K. H.: Oral surgery. New England J. Med., **240**: 721–728, 1949.

560. Thomas, L.: Studies on intravascular thromboplastic effect of tissue suspensions in mice; reaction of mice to intravenous injections of sedimentable tissue component. Bull. Johns Hopkins Hosp., **81**: 1–25, 1947.

561. Thomas, L.: Studies in intravascular thromboplastic effect of tissue suspensions in mice; factor in normal rabbit serum which inhibits thromboplastic effect of sedimentable tissue component. Bull. Johns Hopkins Hosp., **81**: 26–42, 1947.

562. Thompson, W. H.: Die physiologische Wirkung der Protamine und ihrer Spaltungsprodukte. Ztschr. physiol. Chem., **29**: 1, 1900.
563. Thordarson, O.: Hyperprothrombinemia during pregnancy. Nature, **145**: 305, 1940.
564. Thorsin, G.: Dicumarol poisoning with relapse. Lancet, **2**: 420–422, 1947.
565. Thuerer, G. R. and Angevine, D. M.: Influence of Dicumarol on streptococcic infection in rabbits. Arch. Path., **48**: 274–277, 1949.
566. Tidrick, R. T. and Warner, E. D.: Fibrin fixation of skin transplants. Surgery, **15**: 90–95, 1944.
567. Tidrick, R. T., Seegers, W. H. and Warner, E. D.: Clinical experience with thrombin as an hemostatic agent. Surgery, **14**: 191–196, 1943.
568. Tillett, W. S.: The fibrinolytic activity of hemolytic streptococci. Bact. Rev., **2**: 161–216, 1938.
569. Tillett, W. S. and Sherry, S.: The effect in patients of streptococcal fibrinolysin and streptococcal desoxyribonuclease on fibrinous purulent, and sanguinous pleural exudations. J. Clin. Investigation, **28**: 173–190, 1949.
570. Tishler, M. and Evans, H. M.: Vitamin E activities of some compounds related to α-tocopherol. J. Biol. Chem., **139**: 241–245, 1941.
571. Tocantins, L. M.: The mammalian blood platelet in health and disease. Medicine, **17**: 155–260, 1938.
572. Tocantins, L. M.: Cephalin, protamine and antithromboplastic activity of normal and hemophilic plasmas. Proc. Soc. Exper. Biol. & Med., **54**: 94–97, 1943.
573. Tocantins, L. M.: Heparinemia. Blood, **3**: 1304–1306, 1948.
574. Tocantins, L. M. and Carroll, R. T.: Activity curves of crude and purified inhibitors and accelerators of blood coagulation. Proc. Soc. Exper. Biol. & Med., **69**: 431–436, 1948.
575. Troll, M. M. and Menten, M. L.: Salicylate poisoning. Am. J. Dis. Child., **69**: 37–43, 1945.
576. Tudoranu, G., Toporas, P. and Pavelescu, C.: New effect of sulfonamides—role of thiol groups. Le Sang, **20**: 427–429, 1949.
577. Unger, P. N. and Shapiro, S.: Hyperprothrombinemia induced by vitamin K in human subjects with normal liver function. Blood, **3**: 137–146, 1948.
578. Unger, P. N., Weiner, M. and Shapiro, S.: The vitamin K tolerance test. Am. J. Clin. Path., **18**: 835–851. 1948.
579. Used in Japan. (Also in America 20 years ago?)
580. Uvnäs, B.: The plasma prothrombin level in cats and rabbits after excluding the liver from the circulation, after stimulation of the splanchnic nerve and after intravenous injection of adrenalin. Acta physiol. Scandinav., **3**: 97–110, 1941.
581. Vartiainen, I. and Marble, A.: The effect of the subcutaneous administration of protamine (salmine) to rabbits and mice. J. Lab. & Clin. Med., **26**: 1416–1422, 1944.
582. Veit, J.: Ueber Albuminurie in der Schwangerschaft. Ein Beitrag zur Physiologie der Schwangerschaft. Berl. klin. Wchnschr., **39**: 513–516, 1902.
583. Volkert, M.: Studies on the antithrombin content of the blood and its relation to heparin. Acta physiol. Scandinav., Suppl. No. 15, 1942.
584. Vosburgh, C. H. and Richards, A. N.: An experimental study of the sugar content and extravascular coagulation of the blood after administration of adrenalin. Am. J. Physiol., **9**: 35–51, 1903.
585. Wakim, K. G., Chen, K. K. and Gatch, W. D.: The immediate effects of 3,3′-methylenebis (4-hydroxycoumarin) on experimental animals. Surg., Gynec. & Obst., **76**: 323–326, 1943.
586. Wakim, K. G., Fink, R. D. and Chen, K. K.: The influence of adrenalin on plasma prothrombin. Am. J. Physiol., **145**: 452–457, 1946.
587. Walker, J., Jr.: Efficacy of heparin administered by intravenous, intramuscular, and subcutaneous routes and study effect of five bacteriostatic agents on heparin action. Surgery, **17**: 54–60, 1945.
588. Walker, S. E., Eylenburg, E. E. and Moore, T.: The action of vitamin K in hypervitaminosis A. Biochem. J., **41**: 575–580, 1947.
589. Ware, A. G.: Blood coagulation. Univ. South. California Med. Bull., **2**: 20–31, 1950.
590. Ware, A. G. and Seegers, W. H.: Plasma accelerator globulin: Partial purification, quantitative determination and properties. J. Biol. Chem., **172**: 699–705, 1948.
591. Ware, A. G. and Seegers, W. H.: Studies on prothrombin: Purification, inactivation with thrombin, and activation with thromboplastin and calcium. J. Biol. Chem., **174**: 565–575, 1948.
592. Ware, A. G. and Seegers, W. H.: Two-stage procedure for the quantitative determination of prothrombin concentration. Am. J. Clin. Path., **19**: 471–482, 1949.
593. Warner, E. D.: Plasma prothrombin: Effect of partial hepatectomy. J. Exper. Med., **68**: 831–835, 1938.
594. Warner, E. D.: Vitamin K deficiency. M. Clin. North America, **27**: 371–378, 1943.
595. Warner, E. D. and Owen, C. A.: Hypoprothrombinemia in pernicious anemia. Am. J. M. Sc., **203**: 187–191, 1942.
596. Warner, E. D., Brinkhous, K. M. and Smith, H. P.: A quantitative study on blood clotting; prothrombin fluctuations under experimental conditions. Am. J. Physiol., **114**: 667–675, 1936.
597. Warner, E. D., Brinkhous, K. M., Seegers, W. H. and Smith, H. P.: Further experience with the use of thrombin as a hemostatic agent. Proc. Soc. Exper. Biol. & Med., **41**: 655–657, 1939.
598. Warren, S. and Draeger, R. H.: The pattern of injury produced by the atomic bombs at Hiroshima and Nagasaki. U. S. Nav. M. Bull., **46**: 1349–1353, 1946.
599. Weiner, A. E., Reid, D. E. and Roby, C. C.: Coagulation defects associated with premature separation of the normally implanted placenta. Am. J. Obst. & Gynec., **60**: 379–386, 1950.

600. Weiner, A. E., Reid, D. E., Roby, C. C. and Diamond, L. K.: Coagulation defects with intra-uterine death from Rh isosensitization. Am. J. Obst. & Gynec., 60: 1015–1022, 1950.

601. Weiner, L. and Wald, A. H.: Fibrin foam and thrombin as used in the surgical removal of a large fibromyxoma of the mandible. J. Am. Dent. A., 33: 731–735, 1946.

602. Weiner, M., Shapiro, S., Axelrod, J., Cooper, J. R. and Brodie, B. B.: The physiological disposition of Dicumarol in man. J. Pharmacol. & Exper. Therap., 99: 409–420, 1950.

603. Werch, S. C.: Reduction of coagulation time of rabbits' blood by digitalis. Quart. Bull., Northwestern Univ. M. School, 17: 50–51, 1943.

604. Whipple, G. H. and Hurwitz, S. H.: Fibrinogen of the blood as influenced by the liver necrosis of chloroform poisoning. J. Exper. Med., 13: 136–161, 1911.

605. Wier, R. H., Egan, J. C. and Wolfson, S. A.: Effect of Dicumarol on the erythrocyte sedimentation rate. Ann. Int. Med., 33: 354–360, 1950.

606. Wilander, O.: Studien über Heparin. Skand. Archiv. für Phys., Suppl. No. 15, 81: 1–81, 1938.

607. Wilson, S. J.: Differences during Dicoumarol therapy in the Quick and Russell viper venom methods for prothrombin determination. Proc. Soc. Exper. Biol. & Med., 66: 126–128, 1947.

608. Winternitz, M. C., Mylon, E. and Katzenstein, R.: Studies on relation of kidney to cardiovascular disease; tissue extracts and thrombosis. Yale J. Biol. & Med., 13: 595–622, 1941.

609. Wise, W. D., Loker, F. F. and Brambel, C. E.: Effectiveness of Dicumarol prophylaxis against thromboembolic complications following major surgery. A four year survey: 3,304 cases. Surg., Gynec. & Obst., 88: 486–494, 1949.

610. Wöhlisch, E.: Fortschriffe in der Physiologie der Blutgerinnung. Ergebn. d. Physiol., 43: 174–370, 1940.

611. Wolf, A. and Pappenheimer, A. M.: The histopathology of nutritional encephalomalasia of chicks. J. Exper. Med., 54: 399–405, 1931.

612. Wolfrom, M. L. and Karabinos, J. V.: Heparin-hydrolytic characteristics. J. Am. Chem. Soc., 67: 679–680, 1945.

613. Wolfrom, M. L. and McNeely, W. H.: The relation between the structure of heparin and its anticoagulant activity. J. Am. Chem. Soc., 67: 748–753, 1945.

614. Wolfrom, M. L. and Rice, F. A. H.: The uronic acid component of heparin. J. Am. Chem. Soc., 68: 532, 1946.

615. Wolfrom, M. L., Weisblat, D. I., Karabinos, J. V., McNeely, W. H. and McLean, J.: Chemical studies on crystalline barium acid heparinate. J. Am. Chem. Soc., 65: 2077–2085, 1943.

616. Wooldridge, L. C.: On intravascular clotting. Arch. f. Physiol., Leipz., 397–399, 1886.

617. Woolley, D. W.: Some biological effects produced by α-tocopheral quinone. J. Biol. Chem., 159: 59–66, 1945.

618. Wright, H. P.: The adhesiveness of blood platelets in normal subjects with varying concentrations of anticoagulants. J. Path. & Bact., 53: 255–262, 1941.

619. Wright, H. P.: The adhesiveness of blood platelets in rabbits treated with Dicumarol. J. Path. & Bact., 57: 382–385, 1945.

620. Wright, H. P.: Experiences with Dicumarol (3,3′-methylenebis-(4-hydroxycoumarin)) in the treatment of coronary thrombosis with myocardial infarction. Am. Heart J., 32: 20–31, 1946.

621. Wright, I. S.: The modern treatment of coronary thrombosis with myocardial infarction. Circulation, 2: 929–936, 1950.

622. Wright, I. S. and Prandoni, A.: The dicumarin 3,3′-methylenebis (4-hydroxycoumarin): Its pharmacologic and therapeutic action in man. J. Am. M. A., 120: 1015–1021, 1942.

623. Wright, I. S., Marple, C. D. and Beck, D. F.: Anticoagulant therapy of coronary thrombosis with myocardial infarction. J. Am. M. A., 138: 1074–1079, 1948.

624. Wright, I. S., Marple, C. D. and Beck, D. H.: Report of the committee for the evaluation of anticoagulants in the treatment of coronary thrombosis with myocardial infarction. Am. Heart J., 36: 801–815, 1948.

625. Young, F.: Skin graft fixation by plasma-thrombin adhesion. Ann. Surg., 120: 450–462, 1944.

626. Young, F. and Favata, B. V.: The fixation of skin grafts by thrombin-plasma adhesion. Surgery, 15: 378–386, 1944.

627. Young, R. L., Derbyshire, R. C. and Cramer, O. S.: Paradoxical embolism: Review of the literature with report of a case in which this condition followed administration of "Dicumarol". Arch. Path., 46: 43–48, 1948.

628. Zierler, K. L., Grob, D. and Lilienthal, J. L., Jr.: Antithrombic and antiproteolytic activity of α-tocopheryl phosphate. Am. J. Physiol., 153: 127–132, 1948.

629. Zimmerman, S. P. and Shapiro, S.: Correlation between salicylate dosage and plasma level and the prothrombin content of blood. Exper. Med. & Surg., 4: 110–117, 1946.